

Microgeographic genetic heterogeneity of melanic *Daphnia pulex* at a low-Arctic site

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Clonal diversity of obligately parthenogenetic *Daphnia pulex* was examined in 147 ponds at 13 sites near Churchill, Manitoba. Cellulose acetate electrophoresis of four polymorphic loci allowed the detection of 16, multi-locus genotypes (clones). The three most common clones accounted for 86 per cent of the 3291 animals surveyed. An average of 1.5 clones coexisted in single ponds, while an average of 3.8 clones was present at each site. Fifteen of the sixteen clones show unbalanced electromorph phenotypes at one or more loci suggesting that they are polyploids. There was significant spatial autocorrelation of the distribution of certain clones within a given site on a scale of tens of metres. Clinal patterns in clone distributions were often found. No significant between-site spatial autocorrelation was found on a scale of 0–20 kilometres, although between-site heterogeneity of clonal frequencies was frequently seen. Microgeographic heterogeneity of *D. pulex* clones at Churchill is influenced by salinity gradients on a scale of tens of meters. Mechanisms that might influence the clonal structure of this apomictic complex are discussed.

INTRODUCTION

Clonal diversity among parthenogenetic organisms has been well studied (Christensen, 1980; Hebert and Crease, 1980, 1983; Jaenicke *et al.*, 1980; Ochman *et al.*, 1980; Hebert, 1978, 1981; Lynch, 1983; Lyman and Ellstrand, 1984; Rhomberg *et al.*, 1985; Harshman and Futuyma, 1985). Among obligate parthenogens, multiple clones are often present within local populations. For example, Hebert and Crease (1983) observed 22 clones of obligately parthenogenetic *Daphnia pulex* from ponds near Windsor, Ontario. Clonal diversity estimates for a single habitat (pond) ranged from 1–7 clones/pond (mean = 4.0 clones/pond based on 4 enzyme loci). Likewise, Harshman and Futuyma (1985) in a study of the geometrid moth, *Alsophila pometaria*, observed 70 clones in 22 populations scattered across Long Island. Clonal diversity ranged from 2–15 clones/population (mean = 8.4 clones/population based on 4 enzyme loci).

Most studies of clonal diversity in asexuals have involved temperate zone organisms (Bell,

1982). Here we examine the genetic structure of melanic, obligately parthenogenetic *Daphnia pulex* (McWalter, 1981; Hebert and McWalter, 1983) from a low-arctic site. Prior work (Weider and Hebert, 1987) has shown that there is non-random spatial heterogeneity of *Daphnia* clones in rock pools on three quartzite bluffs (bluffs A, B, and C, fig. 1(b),(c) adjacent to Hudson Bay near Churchill, Manitoba. Clonal distributions are associated with the salinity gradients that are related to distance of a pond from Hudson Bay. This study estimates clonal diversity of melanic *D. pulex* at Churchill and examines clonal distribution patterns on a larger scale of tens of kilometres, and on a microspatial scale of tens of metres.

MATERIALS AND METHODS

The study area (fig. 1(a) is located at Churchill, Manitoba (58°47'N, 94°11'W) along a 30 km section of Hudson Bay coast that extends from the west bank of the Churchill River (fig. 1(b), bluff PW) to an area near the National Research Council rocket range (sites C, B, A, EA, and EPA; fig. 1(b).

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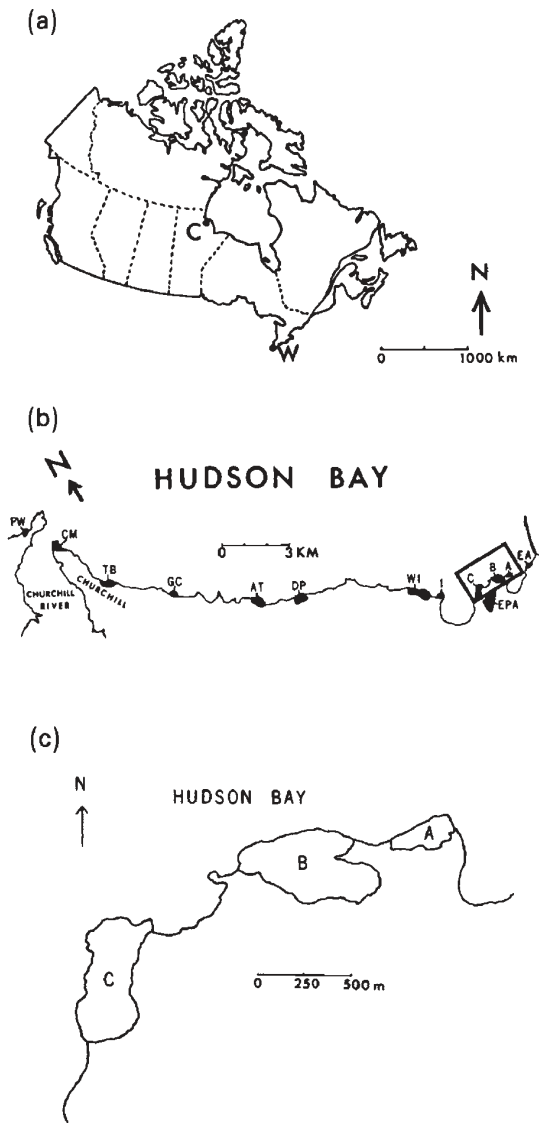


Figure 1 Maps depicting the locations of (a) Churchill, Manitoba (C) and Windsor, Ontario (W); (b) location of sampling sites near Churchill. Abbreviations same as in table 3 legend; (c) map of bluffs A, B, and C that were studied most extensively in 1984, and were used for the within-site spatial analyses.

Melanic *Daphnia pulex* were surveyed electrophoretically in June-July 1985. More extensive collections from bluffs A, B, and C (fig. 1(c)) were made in July 1984 (Weider and Hebert, 1987).

Cellulose acetate electrophoresis (Helena Scientific) was used to determine allozyme phenotypes for individual *Daphnia* at four polymorphic enzyme loci: *PGM* (phosphoglucomutase), *PGI* (phosphoglucose isomerase),

AO (aldehyde oxidase), and *GOT* (glutamate oxaloacetate transaminase). All electrophoretic methods follow Hebert and Payne (1985), and were modified from Harris and Hopkinson (1976). A more detailed outline of the modifications is available from the authors, upon request.

An electrophoretic survey using an additional 17 enzyme loci (10 were polymorphic; *ACON* 1 & 2, *AMY* 1 & 2, *G6PDH*, *HEX*, *LDH*, *MPI*, *PEP-2*, *TPI*, while *FUM*, *G3PDH*, *IDH*, *MDH*, *ME*, *6PGDH*, and *XDH* were monomorphic) examined 118 animals representing 11 four-locus genotypes and failed to detect any additional electrophoretically distinct clones. In previous work (Weider and Hebert, 1987), we report 3 pairs of clones (2 and 2a, 3 and 3a, 4 and 4a) which varied at only one or two additional loci. (Clones 2a, 3a and 4a were detected in the 1984 survey, but were not isolated from the 1985 survey.) Therefore, it would be most accurate to view "clones" as clonal groups that consist of one or several closely-related lineages. Estimates of average genetic distance between clones (Nei, 1972) were calculated using single linkage cluster analysis (BMDP, Dixon and Brown, 1979) based on all 21 enzyme loci. Nei's statistics require gene frequency data which in this case were calculated from the genotypes of individual clones.

Animals were collected from a total of 147 ponds located on 12 rock bluffs and an adjacent section of coastal tundra (EPA, fig. 1(b)), using a 28 × 38 cm (mouth diameter) dip net (200 μm mesh) towed horizontally through the water. No attempt was made to quantify population densities. Ponds were sampled along transects that extended inland from the coast. A total of 3291 animals were sampled in 1985.

Clonal diversity estimates at Churchill were calculated using a random-number generator program on an IBM-PC computer. Presence/absence data for all clones located in all 147 ponds were analysed. The program generated the predicted total number of clones found in single ponds, two-pond combinations, three-pond combinations and so on, up to 147-pond combinations, randomising the pond combinations (100 iterations for each combination). Total number of clones detected vs number of ponds sampled was fitted to a least-squares polynomial regression model.

Spatial autocorrelation analysis (Sokal and Oden, 1978a, b, Cliff and Ord, 1981) was used to determine whether significant spatial patterns exist in clonal distributions, both within and between sampling sites. Within-site spatial autocorrelation was determined for bluffs A, B, and C using data

Table 1 Electromorph banding patterns of melanic *Daphnia pulex* genotypes from Churchill, Manitoba, 1985. Numbers refer to relative mobilities of alleles; i.e., larger number refers to faster (anodally) migrating allele. An * refers to "unbalanced" heterozygotes (see Results section). Two loci were scored for amylase (Amy₁-faster, Amy₂-slower) and aconitase (Acon₁-faster, Acon₂-slower)

Genotype	Enzyme Loci																				
	Pgm	Pgi	Ao	Got	Amy ₁	Amy ₂	Mpi	G3 PDH	Acon ₁	Acon ₂	Fum	Idh	Ldh	G6 PDH	Hex	Me	Mdh ^c	Xdh	Tpi	Pep ₂	6PGDH
1	012*	44	13	22	22	33	23	33	23	22	33	33	13	11	11	33	13*	33	22	11	33
2	23	14*	11	23*	22	13	33	33	23	22	33	33	23	11	22	33	13*	33	22	13	33
3	23	44	22	23*	22	33	33	33	33	22	33	33	13	11	2*3	33	13*	33	22	13	33
4	02*	14*	33	22	23	33	33	33	23	22	33	33	11	22	12	33	13*	33	22	13	33
5	01*2	14*	33	22	23	33	33	33	22	22	33	33	11	22	12	33	13*	33	22	13	33
6	2*4	44	11	22	23	33	34	33	33	33	33	23	11	22	22	33	13*	33	23	13	33
7	22	44	22	23*	22	23	34	33	23	22	33	33	13	11	12	33	13*	33	22	13	33
8	12	44	22	22	33	33	34	33	22	33	33	13	11	11	11	33	13*	33	23	13	33
9	2*4	44	23	23*	23	33	3*4	33	23	33	33	13	11	22	33	33	13*	33	23	13	33
10	23	44	33	23*	23	33	33	33	23	22	33	33	11	11	2*3	33	13*	33	23	13	33
11	22	14*	44	22	23	33	3*4	33	23	22	33	33	11	11	12	33	13*	33	23	13	33
12	23	14*	22	23*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— [@]
13	2*4	14*	13	23*	23	33	45	33	22	22	33	33	13	11	12	33	13*	33	23	11	33
14	02*	44	33	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15	23	44	11	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	123*	44	13	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

^c Supernatant form of *Mdh* (malate dehydrogenase).

[@] Unavailable for analysis, no laboratory stock culture. *Unique allele (1⁺) that was slightly faster than allele 1. Enzyme abbreviations as follows: *Pgm* = phosphoglucomutase, *Pgi* = phosphoglucose isomerase, *Ao* = aldehyde oxidase, *Got* = glutamate oxaloacetate transaminase, *Amy*_{1,2} = amylase, *Mpi* = mannose phosphate isomerase, *Acon*_{1,2} = aconitase, *Fum* = fumarase, *Idh* = isocitrate dehydrogenase, *Ldh* = lactate dehydrogenase, *G-6PDH* = glucose-6-phosphate dehydrogenase, *Hex* = hexokinase, *Me* = malic enzyme, *Xdh* = xanthine dehydrogenase, *Tpi* = triose phosphate isomerase, *Pep*₂ = peptidase phenylalanyl-leucine, *G-3PDH* = 6-phosphogluconate dehydrogenase, *G-3PDH* = glyceraldehyde-3-phosphate dehydrogenase.

collected in 1984 (Weider and Hebert, 1987). The within-site analysis was restricted to the three most extensively-studied sites. For the between-site analyses, the within-site data were pooled for each bluff. Both within and between-site data were analysed using Spatial Autocorrelation Analysis Program (SAAP, Wartenberg, 1985) on an IBM-PC computer. Spatial autocorrelation coefficients (Moran's I) were determined for the clonal frequencies of the most abundant clones, as a function of distance between sampling sites. Distances were calculated from aerial photographs (National Air Photo Library, Ottawa) using a Bioquant digitiser pad interfaced with an Apple computer. Coordinate (x, y) data were generated for each site (mid-bluff point), as well as for individual ponds on bluffs A, B, and C. Distance class intervals were computer-generated, so that a minimum of 30–40 distance pairs were distributed in each distance class, as suggested by Wartenberg (1985).

RESULTS

Clonal diversity

Sixteen clones were detected on the basis of the electrophoretic variation at four loci (*PGM*, *PGI*, *AO*, *GOT*; table 1). The three most common clones (clones 1, 3 and 4) accounted for 86 per cent ($N = 2817$) of the animals surveyed ($N = 3291$ table 2). Between-site differences in clonal frequencies (table 3) were often seen. Clone 3 was the most common clone and was found on all 12 bluffs, but was absent from the EPA tundra ponds. Clone 4 was also absent from the EPA, but was present

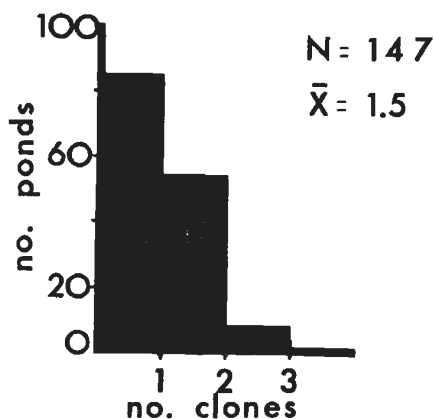


Figure 2 Histogram showing the number of clones detected in each of 147 ponds. Mean number of clones/pond was approximately 1.5.

Table 2 Summary of clonal frequencies of melanic *Daphnia pulex*, 1985

Clone No.	N =	% of total	No. of ponds
1	674	20.5	40
2	109	3.3	10
3	1475	44.8	82
4	668	20.3	43
5	21	0.6	3
6	72	2.2	6
7	48	1.5	4
8	4	0.1	2
9	120	3.6	11
10	42	1.3	8
11	20	0.6	2
12	2	0.05	1
13	32	1.0	2
14	2	0.05	1
15	1	0.05	1
16	1	0.05	1
Total	3291	100.00	

in high-salinity ponds near the seaward-side on 10 of 12 bluffs. Clone 1 was found on 7 of 12 bluffs, but was restricted to low-salinity ponds on the landward-side of the bluffs. Clone 1 was very abundant in the EPA tundra ponds. Clone 9 was found on 6 of 12 bluffs, but was not detected at any site east of Ithica Bluff (I). Rare clones (<2 per cent of the total sample) were often restricted to a single site (clones 11–16, table 3). Mean clonal diversity at a given site (bluff) was 3.8 clones/site, while mean clonal diversity in a given pond was 1.5 clones/pond (fig. 2).

Allozyme phenotypes

Six alleles were found at the *PGM* locus, four at *AO*, and two at *PGI* and *GOT*. The homozygotes at *PGI* were single-banded, while all heterozygotes at *PGI* were triple-banded, as expected on the basis of the dimeric structure of the enzyme. However, the relative banding intensities did not conform to the expected 1:2:1 ratio. Instead, the staining levels approximated a 9:6:1 ratio. Similar "unbalanced" heterozygotes were observed at *GOT*. *PGM* is a monomeric enzyme and heterozygotes ordinarily show two equally staining bands. However, 8 of 14 heterozygotes showed greater activity of one allele product, while several clones (1, 5, 16, Table 1) showed triple-banded phenotypes. The supplemental survey of electrophoretic variation (table 1) revealed "unbalanced" heterozygotes at three additional loci, *HEX*, *MDH*, and *MPI*.

Table 3 Clonal frequencies of melanic *Daphnia pulex* from Churchill, Manitoba, 1985. Sample sizes given in parentheses. Site abbreviations: *PW* = Prince of Wales (11 ponds); *CM* = Cape Merry (12); *TB* = Town Bluff (20); *GC* = Golf Course (11); *AT* = Airport (7); *DP* = Dump (2); *WI* = West Ithica (6); *I* = Ithica (10); *C* = Bluff C (15); *B* = Bluff B (16); *A* = Bluff A (20); *EA* = East A (10); *EPA* = Experimental Ponds Area (7)

Clone No.	Sampling Sites												
	<i>PW</i>	<i>CM</i>	<i>TB</i>	<i>GC</i>	<i>AT</i>	<i>DP</i>	<i>WI</i>	<i>I</i>	<i>C</i>	<i>B</i>	<i>A</i>	<i>EA</i>	<i>EPA</i>
1		0.015 (4)	0.202 (98)	0.364 (96)					0.319 (107)	0.466 (145)	0.265 (96)	0.104 (25)	0.665 (103)
2											0.301 (109)		
3	0.784 (207)	0.442 (118)	0.324 (157)	0.610 (161)	0.702 (118)	0.479 (23)	0.516 (79)	0.297 (71)	0.501 (168)	0.328 (102)	0.169 (61)	0.875 (210)	
4	0.186 (49)	0.416 (111)	0.327 (159)	0.004 (1)		0.521 (25)	0.320 (49)	0.598 (143)	0.090 (30)		0.265 (96)	0.021 (5)	
5									0.063 (21)				
6									0.024 (8)	0.206 (64)			
7													0.310 (48)
8													0.025 (4)
9	0.007 (2)		0.031 (15)	0.018 (5)	0.298 (50)		0.150 (23)	0.105 (25)					
10	0.023 (6)		0.074 (36)										
11			0.042 (20)										
12							0.014 (2)						
13		0.120 (32)											
14		0.007 (2)											
15									0.003 (1)				
16				0.004 (1)									
No. of clones	4	5	6	5	2	2	4	3	6	3	4	3	3

Estimating clonal diversity and relatedness

Fig. 3 shows that the relationship between number of clones detected and the number of ponds sampled best approximated a power function represented by the equation

$$Y(\text{clones}) = 2.17X(\text{ponds})^{0.41}$$

($R^2 = 0.98$). Seventy-five per cent (12/16) of the detected clones were found in the first 60–70 ponds that were sampled.

Mean genetic distances (fig. 4) between clones (including clones 2a, 3a and 4a; Weider and Hebert, 1987) ranged from 0.01–0.25 with a mean value (+/- 1 S.E.) of 0.11 +/- 0.02. (Clones 12, 14, 15 and 16 were not included in the analysis because laboratory cultures of these rare clones were not isolated). These data indicate a level of genetic differentiation that is intermediate between

conspecific populations and “typical sibling species” (Nei, 1972). These estimates of relatedness of clones are relative estimates based on the 21 loci chosen, and are not absolute values. Additional dendrograms might provide an equally acceptable statement of genetic relatedness (Felsenstein, 1985).

Spatial heterogeneity

Spatial autocorrelation analyses of the within-site clonal frequency data showed that there is significant spatial patterning of certain clones (table 4, fig. 5). On Bluff A, clones 1, 2 and 4 showed significant positive spatial autocorrelation in the smallest distance class (0–24 m), indicating that ponds in this distance class were more similar in clonal frequencies than ponds outside the distance

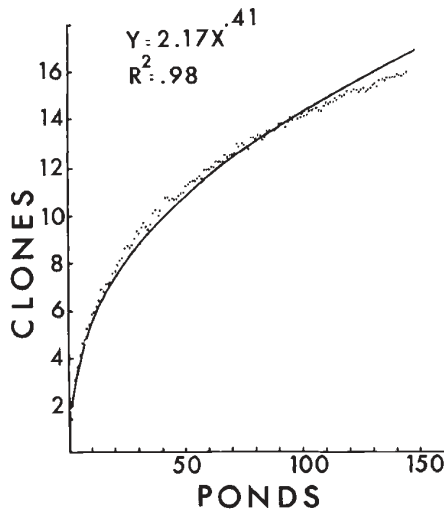


Figure 3 Results of computer analysis showing the relationship between total number of clones detected (Y) and a random sample of different pond combinations (X). The relationship best approximates a power function of $Y = 2.17X^{0.41}$.

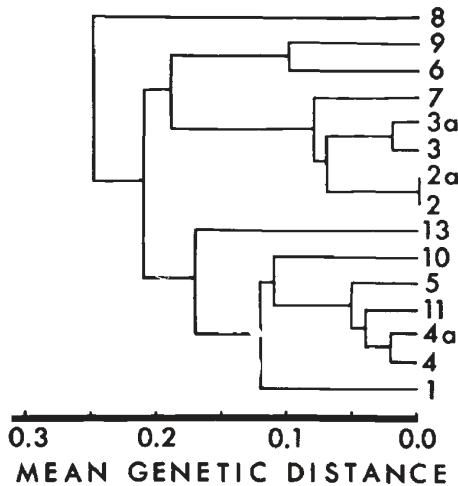


Figure 4 Mean genetic distance dendrogram for melanic *Daphnia pulex* clones. Numbers (1, 4, 4a, ...) refer to clones.

class. At larger distances (fig. 5, table 4), the clonal patterns switch to significant negative autocorrelation especially in the largest class (120-144 m). Clone 4 exhibited a clinal pattern to its distribution on Bluff A (fig. 5(a)). Interestingly, clone 3 exhibited no significant spatial autocorrelation on Bluff A (fig. 5(a), table 4), but exhibited strong clinal patterns on Bluffs B and C as did clone 1 (fig. 5(b), (c)). The lack of significant autocorrelation for clone 3 on bluff A may be a consequence

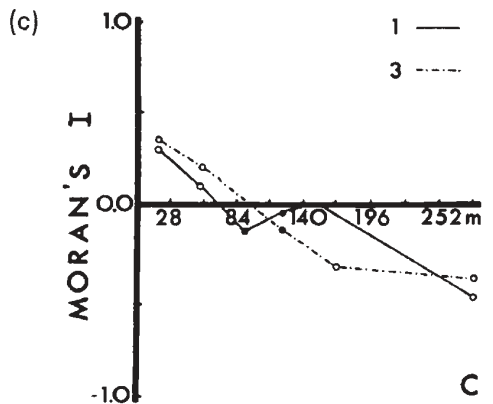
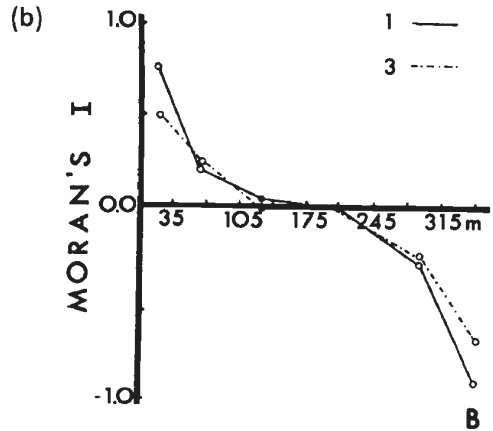
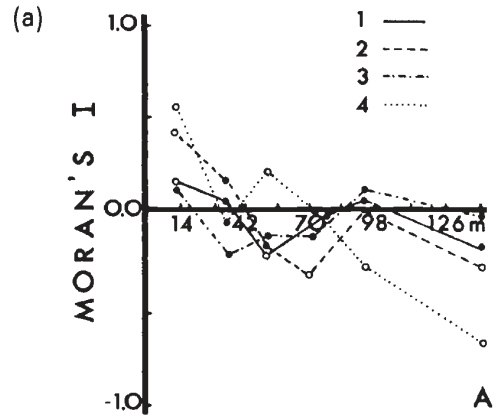


Figure 5 Spatial correlograms depicting the autocorrelation coefficients (Moran's I) as a function of distance between ponds for clonal frequencies of the most common clones (numbers refer to clones) on bluffs A (a), B (b), and C (c). Open circles = significant values (see Table 4). Closed circles = non-significant values.

Table 4 Spatial autocorrelation statistics (Moran's I) for the clonal frequency data at Churchill, Manitoba collected in 1984 on Bluffs A, B, and C. Within-site spatial patterns were examined

Bluff	Clone No.	Distance class					
		0-24 m	24-48	48-72	72-96	96-120	120-144
A	1	0.151 ^a	0.087	-0.218 ^a	-0.053	0.040	-0.127
	2	0.389 ^f	0.140	-0.169	-0.287 ^c	-0.021	-0.248 ^c
	3	0.092	-0.200	-0.097	-0.077	0.109	-0.013
	4	0.542 ^f	-0.035	0.190 ^b	-0.021	-0.226 ^a	-0.646 ^f
B		0-60 m	60-120	120-180	180-240	240-300	300-360
	1	0.774 ^f	0.220 ^f	0.062	0.000	-0.259 ^c	-0.924 ^f
	3	0.523 ^f	0.246 ^f	0.013	-0.014	-0.218 ^d	-0.675 ^f
C		0-50 m	50-100	100-150	150-200	200-250	250-300
	1	0.303 ^f	0.122 ^a	-0.077	-0.026	-0.012	-0.457 ^f
	3	0.354 ^f	0.210 ^d	0.021	-0.084	-0.301 ^c	-0.345 ^f

^a $P < 0.05$. ^b $P < 0.02$. ^c $P < 0.01$. ^d $P < 0.001$. ^e $P < 0.0001$. ^f $P < 0.00002$.

of a scale effect due to the small size of bluff A (total distance on bluff A of approximately 150 m, as compared to 350 m and 280 m on bluffs B and C, respectively).

An examination of between-site clonal frequencies indicated that each of the most common clones (1, 3 and 4) exhibited significant heterogeneity of mean frequencies (G -test, $G = 341, 196, 306$ respectively; $P < 0.001$). However, when between-site spatial autocorrelation is examined for the three clones on all bluffs, no consistent spatial patterns were observed (table 5).

Table 5 Spatial autocorrelation statistics (Moran's I) for between-site clonal frequency data collected at Churchill, Manitoba in 1985

Clone No.	Distance class			
	0-3.0 km	3.0-9.2	9.2-16.2	16.2-22.2
1	0.059	-0.267	0.113	-0.290
3	-0.094	-0.056	-0.082	-0.249
4	-0.138	-0.290	0.264	-0.196

DISCUSSION

The electrophoretic studies indicated that melanic *Daphnia pulex* at Churchill exhibited unusual allozyme phenotypes. Specifically, all of the clones showed "unbalanced" heterozygote phenotypes at one or more loci, and several clones exhibited three-banded heterozygotes at the monomeric *PGM* locus. Such phenotypes suggest that these clones are polyploids, and DNA-quantification studies (Hebert, 1986; M. Beaton, pers. comm.) have indicated that all the clones included in this survey are tetraploids (4n).

The clonal diversity surveys at Churchill revealed an average of 1.5 clones/pond, with a total of 16 clones detected from the Churchill area. These 16 clones showed considerable differences in both relative abundance (table 2), as well as in distributions (table 3). Generally, there are about 3 clones/pond in obligately asexual populations of *D. pulex* from sites in both southern Ontario and the western arctic (Hebert and Crease, 1980, 1983; Hebert and Loaring, 1986). An analysis of 65 populations in southwestern Ontario revealed 36 clones (Hebert *et al.*, in preparation). On this basis, melanic *D. pulex* populations at Churchill are considerably less clonally diverse than other populations of obligately parthenogenetic *D. pulex*, both on a microscale (single pond) and localised scale (tens of kilometres).

The present study has clearly not identified all clones in the Churchill area. As mentioned above, three clones (2a, 3a and 4a; fig. 4) are closely related to clones 2, 3 and 4, respectively, and may represent mutationally-derived lineages. Additional "mutationally-derived" clones would undoubtedly be detected if more loci were surveyed. Our clonal diversity estimates at Churchill are in this sense conservative. To detect additional independently-derived lineages in the Churchill area would not be easy.

The source of clonal diversity in this apomictic complex remains unclear. Parker (1979) considers some of the ecological and evolutionary implications of multiclonal "morphospecies" and concludes that their mode of origin as mono- or polyphyletic groups is important in determining the nature of the selective forces operating on clonal arrays. Clones that arise through polyphyletic origins from the same sexual species differ at many loci, and may differ in fitness attributes such

as life history characters. Such clones may be adapted to different microhabitats, and may have reduced niche overlap. In contrast, mutationally-derived clones from a single ancestral lineage with a monophyletic origin may initially show no fitness or ecological differences because single gene differences may not be expressed phenotypically. The genetic divergence among clones found at Churchill is compatible with a polyphyletic origin. The loss of sex in the Churchill *D. pulex* complex is probably the result of sex-limited suppressors of meiosis (Hebert, 1981, 1986; Innes and Hebert, in review), and such suppressors provide a mechanism for the polyphyletic loss of sex.

Spatial patterning of clones showed significant within-site spatial autocorrelation on a scale of tens of metres (table 4; fig. 4). There is in addition an association between salinity gradients and clonal distributions of melanic *D. pulex* at Churchill. Differences in salinity tolerance between clones suggest that this *Daphnia* "complex" has differentiated into physiologically distinct ecotypes (Weider and Hebert, 1987). Within-site variation in clonal distributions are influenced strongly by spatial patterning of selective forces (i.e., salinity gradients). Additional selective factors such as invertebrate predators may also be important (personal observation). Both within-site and between-site spatial patterns imply that patterning of selective factors is on a much smaller scale than inter-site distance (tens of metres versus kilometres). This might explain why no consistent between-site clonal patterns were found (table 5).

Although no significant between-site autocorrelation was detected (scale of 0–20 km; table 5), there was considerable between-site heterogeneity of clones. This spatial heterogeneity is probably related to environmental heterogeneity. For example, clone 1 was found at a lower frequency on bluff A (table 3), than on bluffs B and C, perhaps because of the higher salinity of pond water on bluff A (2390 ± 558 µS/cm on A; 469 ± 68 µS/cm on B; 1208 ± 246 µS/cm on C). However, other distributional patterns are more difficult to explain. For instance, clone 2 was found in 10 of 20 ponds on bluff A (table 3), but was not detected on any other bluff. Additional environmental factors that influence pond water quality such as nutrient input may be involved.

If clonal diversity patterns at Churchill are in equilibrium, then present levels of clonal diversity are a product of the interaction between diversity-generating and diversity-reducing processes. Diversity may be increased by the dispersal of new clones into the region, and by the creation of new

clones *in situ*. Such new clones might arise from the mutational divergence of existing lineages, or by the formation of new lineages. Despite the absence of cyclically parthenogenetic lines of *D. pulex* in the Churchill area, a number of melanic (polyploid) and non-melanic (diploid) obligately parthenogenetic clones have retained the ability to produce males (personal observations). It is possible that polyploid melanic clones may have been produced through hybridisation between diploid clones resulting in the present clonal array.

The elucidation and measurement of diversity-reducing processes such as competitive exclusion, differential predation, and elimination of clones (through stochastic forces) requires further research. The maintenance of clonal diversity in the Churchill region appears to be linked to spatial (and to a lesser extent temporal) heterogeneity in the environment. Further study of the processes that maintain clonal diversity and influence clonal distributions is needed.

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