Genetic diversity and breeding system variation in *Daphnia pulicaria* from North American lakes

MARTIN ČERNÝ* & PAUL D. N. HEBERT†

Department of Hydrobiology, Charles University, Prague, CS-128 44, Czech Republic; †Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Comparative distributional studies of closely related taxa suggest that sexuals dominate in stable habitats, while asexuals are most abundant in disturbed environments. For freshwater zooplankton, temporary ponds represent unstable systems in which populations must be re-established from diapausing eggs, while lakes are relatively stable habitats permitting populations to persist all year round. This study involves the comparison of breeding systems in populations of the zooplankter *Daphnia pulicaria* from lake and pond habitats. In common with other members of the genus, some populations of this species have made the transition from cyclic parthenogenesis, in which sexual reproduction is a regular part of the life cycle, to obligate parthenogenesis. Prior work has shown that populations of *D. pulicaria* from ponds in central Canada largely reproduce by obligate parthenogenesis. However, this study shows that cyclic parthenogenesis is the dominant mode of reproduction in lake populations of *D. pulicaria* in two regions of the USA. Obligately asexual lake populations were more frequent in the western than central states, a pattern which may be due to regional variation in the extent of interspecific gene flow from the closely related species *D. pulex*.

Keywords: allozymes, breeding systems, *Daphnia*, parthenogenesis, zooplankton.

Introduction

Cyclic parthenogenesis is both the dominant and ancestral mode of reproduction in cladoceran crustaceans, but some species have made the transition to obligate asexuality (Hebert, 1987). In such taxa both subitaneous and resting eggs are produced asexually, while in cyclically parthenogenetic species the resting eggs are produced sexually. The most detailed analysis of factors promoting this breeding system transition has involved the analysis of *Daphnia pulex* Linne emend. Levdig and its closest relative *D. pulicaria* Forbes. These studies (Hebert *et al.*, 1989a; Lynch *et al.*, 1989; Weider & Hebert, 1987; Weider *et al.*, 1987; Hebert *et al.*, 1993) have demonstrated that both species show regional and local variation in their breeding systems.

This past work has focused on populations from shallow ponds, as breeding systems can be diagnosed with confidence in these settings, because populations are regularly reinitiated from resting eggs. Obligately asexual populations show low genotypic diversity and

gross disturbances from Hardy Weinberg (H. W.) equilibrium, while populations reproducing by cyclic parthenogenesis show high genotypic diversity and the congruence of genotypic frequencies to H. W. expectations. Prior studies have shown that short bouts of asexual reproduction following re-establishment from resting eggs do not have substantial impacts on the genotypic composition of cyclically parthenogenetic populations (Hebert, 1987), ensuring that breeding systems can be reliably diagnosed from the analysis of allozyme markers.

Much less work has been directed towards the analysis of breeding system diversity in lake daphniids because of the potential for interpretational uncertainty. These difficulties arise because work on pond daphniids has shown that when sexual recruitment is rare and the opportunity exists for sustained reproduction via subitaneous eggs, then cyclically parthenogenetic populations can develop genetic characteristics which mimic those of obligate asexuals (Hebert, 1978). It was presumed that similar H. W. disturbances and low genotypic diversity would be the rule in cyclically parthenogenetic populations from lake environments, where there is no recurrent need to re-establish

*Correspondence.

populations from resting eggs. The genetic data now available challenge this conclusion and show that genotypic frequencies are often in H. W. equilibrium (Mort & Wolf, 1985; 1986). Obviously sexual recruitment is more pervasive in lake populations of *Daphnia* than was initially supposed. However, H. W. disturbances have been detected in enough populations known to reproduce by cyclic parthenogenesis (Hebert *et al.*, 1989b; Jacobs, 1990) to make it clear that H. W. deviations can reflect either the adoption of obligate asexuality or the suppression of sexual recruits in a cyclic parthenogenetic population. Fortunately, a similar interpretational uncertainty does not apply to the converse situation — when genotypic diversity is high and frequencies approximate H. W. expectations, it can reasonably be concluded that the population under study reproduces via cyclic parthenogenesis.

This study examines patterns of genetic diversity in populations of *D. pulicaria* from lakes in two regions of temperate North America. Prior work has shown that most populations of this species from pond habitats in central Canada are obligate asexuals, while those in far western Canada are cyclic parthenogens (Hebert *et al.*,

1993). The present study employs allozyme analysis to discover if there is a similar pattern of breeding system variation in lake settings.

Materials and methods

Populations of *D. pulicaria* were sampled from 55 permanent waterbodies (Table 1) comprising both lakes and reservoirs at sites across Ontario and 11 US states. The type locality (Yellowstone Lake) for the species (Hrbáček, 1957) was included among these sites. These populations were subdivided into a group of 19 populations from six western states and a group of 36 populations from five central states and Ontario. Out of the group of 36 populations, 23 were obtained from lakes in north-eastern Indiana, permitting a particularly detailed analysis of variation in breeding systems at this locality.

Sites were sampled in the spring and early summer of 1990 and 1991, shortly after the recruitment of individuals from resting eggs was likely to reach its peak. Samples were ordinarily collected by towing a plankton net in the deepest part of each lake, but a few

Table 1 List of North American lakes classified by region and state where genetic diversity and breeding system variation in *Daphnia pulicaria* was investigated

Region	State	Code	Population (county)
Central	Indiana	in1	Adams Lake (LaGrange)
		in2	Bear Lake (Noble)
		in3	Big Lake (Noble)
		in4	Blue Lake (Whitley)
		in5	Cedar Lake (Whitley)
		in6	Crane Lake (Noble)
		in7	Crooked Lake (Whitley/Noble)
		in8	Eagle Lake (Noble)
		in9	Fish Lake (LaGrange)
		in10	High Lake (Noble)
		in11	Little Cedar Lake (Whitley)
		in12	Little Crooked Lake (Whitley/Noble)
		in13	Loon Lake 1991 (Noble/Whitley)
		in14	Loon Lake 1990 (Noble/Whitley)
		in15	Old Lake (Whitley)
		in16	Oliver Lake (LaGrange)
		in17	Round Lake (Whitley)
		in18	Smalley Lake (Noble)
		in19	Sylvan Lake (Noble)
		in20	Tippecanoe Lake (Kosciusko)
		in21	Waubee Lake 1991 (Kosciusko)
		in22	Waubee Lake 1990 (Kosciusko)
		in23	Wawasee Lake 1991 (Kosciusko)
		in24	Wawasee Lake 1990 (Kosciusko)
		in25	Westler Lake (LaGrange)
		in26	Winona Lake (Kosciusko)

Table 1 Continued

Region	State	Code	Population (county)
Central	Iowa	io1	Storm Lake (Buena Vista)
		io2	Lost Island Lake (Palo Alto)
Central	Michigan	mi1	Cedar Lake (Van Buren)
		mi2	Hemlock Lake (Cass)
		mi3	Silver Lake at Wolverine (Cheboygan)
Central	Minnesota	mn1	Maple Lake (Itasca)
		mn2	Swan Lake (Itasca)
Central	Ontario	on1	Haynes Lake (York)
		on2	Lake at Rossport (Thunder Bay)
Central	Wisconsin	wi1	Kegonsa Lake (Dane)
		wi2	Lake Mendota May 1991 (Dane)
		wi3	Lake Mendota July 1991 (Dane)
		wi4	Lake Monona (Dane)
		wi5	Lake Waubesa (Dane)
West	California	ca1	Lucerne Lake (Mateo)
		ca2	Shasta Lake (Shasta)
		ca3	Whiskeytown Lake (Shasta)
West	Colorado	co1	Cucharas Reservoir (Huefrano)
		co2	Granby Lake (Grand)
		co3	Lake Meredith (Crowley)
		co4	Lake at Pagosa Springs (Archuleta)
West	Idaho	id1	Palisades Reservoir (Bonneville)
West	Nevada	ne1	Jiggs Reservoir (Elko)
		ne2	Lahontan Reservoir (Lyon/Churchill)
		ne3	Washoe Lake (Washoe)
West	Utah	ut1	Deer Creek Reservoir (Wasatch)
		ut2	Koosharem Reservoir (Sevier)
		ut3	Otter Creek Reservoir (Piute)
		ut4	Starvation Lake (Duchesne)
West	Wyoming	wy1	Big Sand Reservoir (Sublette)
		wy2	Lewis Lake (Yellowstone)
		wy3	Soda Lake (Sublette)
		wy4	Yellowstone Lake (Yellowstone)

lakes were sampled from shore. All samples were sorted in the field and when possible at least 60 individuals of *D. pulex* or *D. pulicaria* were frozen in liquid nitrogen.

Although *D. pulex* and *D. pulicaria* are morphologically distinct, their discrimination is complicated by the occurrence of F_1 hybrids. However, past studies have revealed fixed allozyme differences which can be used in species diagnosis (Hebert *et al.*, 1989a). In this study all individuals homozygous for the *F* allele at the LDH locus were classified as *D. pulicaria*, while individuals homozygous for the *S* allele were categorized as *D. pulex*. Individuals heterozygous at LDH (lactate dehydrogenase) were presumed to be F_1

hybrids. Populations assigned to *D. pulicaria* ordinarily contained only individuals with a head shape similar to that of *D. pulicaria* (Brandlová *et al.*, 1972, = *D. schoedleri* sensu Brooks, 1957). It is possible that some individuals categorized either as hybrids or *D. pulex* represent *D. pulicaria* introgressants, but exclusion of this possibility requires more detailed analysis of the populations containing such individuals. It is worth noting that the mistaken exclusion of true introgressants has no effect on the breeding system diagnosis. By contrast, their retention, if they represent reproductively isolated units, would enhance the incidence of H. W. disturbances and hence exaggerate the incidence of asexual populations.

To assess the genotypic structure of each population, at least 40 individuals were scored for allozyme variation at both *LDH* and six additional loci (Aldehydeoxidase, *AO*; Fumerase, *FUM*; Glutamate-oxaloacetate transferase, *GOT*; Mannose phosphate isomerase, *MPI*; Phosphoglucose isomerase, *PGI*; Phosphoglucumutase *PGM*) which are commonly polymorphic in the *D. pulex* complex. Alleles were assigned a letter code (*B* = slowest, *S* = slow, *M* = medium, *F* = fast and *Q* = fastest). All electrophoresis was carried out on Titan III cellulose acetate plates employing standard methods (Hebert & Beaton, 1989). In most cases all 40 individuals were assigned, on the basis of *LDH* phenotype, to *D. pulicaria*. In other cases sample size was reduced by the presence of *D. pulex* or hybrids. The gene frequency data on *D. pulicaria* were employed as a basis for two analyses. The extent of gene frequency divergence among populations was evaluated using Wright's (1978) hierarchical *F*-statistics approach and calculated using BIOSYS-1 (Swofford & Selander, 1989). The significance of *F* values for each hierarchical level was subsequently tested using the χ^2 analysis developed by Workman and Niswander (1970). The gene frequency data were subsequently pooled for all populations from each province or state and Nei's (1978) unbiased genetic distances were calculated for each group of populations. The genetic relatedness of these groups of populations was later evaluated using the unweighted pair-group clustering method in BIOSYS-1.

Fifty-two populations of *D. pulicaria* were represented by sufficient individuals (>15) to permit breeding system assignments. The breeding systems of two populations were classified as uncertain because they were monomorphic at each of the six loci. The remaining populations were polymorphic at one or more loci, and three parameters useful in breeding system determination were calculated for each of these populations: the number of multi-locus genotypes identified, the log-transformed probability of the observed genotypic array at each polymorphic locus being in H. W. equilibrium, and the genotypic diversity ratio (GDR: the observed number of multi-locus genotypes in the sample divided by the expected number in a similarly sized sample from a sexual population with the same gene frequencies).

These three parameters were calculated using the methods described in Hebert *et al.* (1988), and decisions concerning the breeding system were based on rules employed by Hebert *et al.* (1993): i.e. populations were designated as cyclic parthenogens when the probability that genotypic frequencies were in H. W. equilibrium was greater than 5 per cent and the GDR was at least 0.70. Populations were assigned as obligate

parthenogens when genotypic frequencies deviated significantly ($P < 0.05$) from H. W. equilibrium and when the GDR was lower than 0.70. Populations lacking significant deviations from H. W. equilibrium but with a low GDR (<0.70) were designated as possessing an uncertain breeding system, as were those significantly deviant from H. W. equilibrium, but with a high GDR (>0.70).

Results

Taxon distributions

Based on the analyses of *LDH* phenotypes, *D. pulicaria* comprised more than 91 per cent of the 2268 individuals from the 55 habitats. *D. pulicaria* was the sole species at 45 of these sites and dominated at six of the remaining localities (Table 2). In five lakes either *D. pulex* or hybrids co-occurred with *D. pulicaria*, while at the other 5 localities all three taxa co-occurred. At one of the sites (Lake Mendota) there was some evidence for temporal variation in abundances of the taxa. Morphological comparison of individuals assigned to each of three taxa at this site indicated that differences in head shape were small or absent among them.

Gene frequency variation

Four of the six loci commonly polymorphic in members of the *D. pulex* complex (Hebert *et al.*, 1993) were invariant in most lake populations of *D. pulicaria* (Table 3). All populations were fixed for the *M* allele at *FUM* and all except two populations from Nevada (ne2 and ne3) were monomorphic for the *F* allele at *AO*. (The data for *FUM* and *AO* loci are not shown in Table 3.) The same lack of variation was noted at *GOT*

Table 2 Proportions of *Daphnia pulex*, hybrids and *D. pulicaria* at 10 localities where two or more taxa were found to co-occur (px, *D. pulex*; F₁, hybrids; pul, *D. pulicaria*)

	<i>n</i>	px	F ₁	pul
Co, Pagosa Springs	40	0.00	0.57	0.42
Io, Lost Island	40	0.85	0.03	0.13
In, Wawasee 90	38	0.03	0.00	0.97
Mn, Maple	40	0.03	0.00	0.98
Ne, Washoe	39	0.08	0.26	0.67
On, Haynes	40	0.00	0.87	0.13
Wi, Kegonsa	55	0.00	0.09	0.91
Wi, Mendota May	40	0.18	0.17	0.65
July	40	0.03	0.45	0.53
Wi, Monona	40	0.03	0.05	0.92
Wi, Waubesa	39	0.59	0.03	0.38

Table 3 Allelic frequencies and genotypic characteristics at four of the five polymorphic loci analysed in populations of *Daphnia pulicaria* from North American lakes

Population	n	PGI				PGM				GOT				MPI				GDR	bm
		B	S	M	F	Q	B	S	M	F	M	F	B	S	M	F	G		
ca1	40	0	0.48	0.53	0	0	0	0	1	0	0.95	0.05	0	0	1	0	2	0.40	O
ca2	26	0	0.31	0.68	0	0.01	0	0.10	0.89	0.02	0.91	0.09	0	0	0.99	0.01	10	0.78	C
ca3	19	0	0.50	0.50	0	0	0	0	1	0	0.92	0.08	0	0	1	0	2	0.40	O
California	85	0	0.43	0.57	0	0	0	0.03	0.96	0.01	0.93	0.07	0	0	1				
co1	28	0	0.02	0.81	0.17	0	0	0.05	0.95	0	1	0	0	0	1	0	4	0.82	C
co2	39	0	0.28	0.73	0	0	0	0.30	0.71	0	1	0	0	0	1	0	4	0.56	O
co3	40	0	0	1	0	0	0.48	0.08	0.45	0	1	0	0	0	1	0	3	0.59	O
co4	17	0	0	0.85	0.15	0	0.47	0.03	0.18	0.32	1	0	0	0	1	0	5	0.59	U
Colorado	124	0	0.09	0.85	0.06	0	0.22	0.13	0.61	0.04	1	0	0	0	1	0			
id1	39	0	0.09	0.90	0.01	0	0	0	1	0	1	0	0	0	0.99	0	4	1.09	C
Idaho	39	0	0.09	0.90	0.01	0	0	0	1	0	1	0	0	0	0.99	0			
in1	40	0	0.14	0.30	0.56	0	0	0	0.26	0.74	1	0	0	0	1	0	11	0.92	C
in2	39	0	0	0.78	0.23	0	0	0	0.86	0.14	1	0	0	0	1	0	7	1.19	C
in3	38	0	0	0.26	0.74	0	0	0	1	0	1	0	0	0	1	0	3	1.02	C
in4	41	0	0	0.92	0.09	0	0	0	0.82	0.14	1	0	0	0	1	0	3	0.59	U
in5	41	0	0	0.99	0.01	0	0	0	0.52	0.48	1	0	0	0	0.98	0.02	3	0.58	O
in6	40	0	0	0.25	0.75	0	0	0	1	0	1	0	0	0	1	0	3	1.03	C
in7	40	0	0	0.31	0.69	0	0	0	0.90	0.10	1	0	0	0	1	0	5	0.87	C
in8	40	0	0	0.70	0.30	0	0	0	0.24	0.76	1	0	0	0	1	0	3	0.41	O
in9	40	0	0.34	0.15	0.51	0	0	0	0.59	0.41	1	0	0	0	1	0	6	0.45	U
in10	32	0	0	1	0	0	0	0.02	0.75	0.23	1	0	0	0	1	0	4	1.12	C
in11	41	0	0	0.95	0.05	0	0	0	0.77	0.23	1	0	0	0	1	0	4	0.82	C
in12	40	0	0	0.09	0.91	0	0	0	0.91	0.09	1	0	0	0	1	0	3	0.73	C
in13	40	0	0	0.46	0.54	0	0	0	0.94	0.06	1	0	0	0	1	0	5	0.93	C
in14	33	0	0	0.92	0.08	0	0	0	0.34	0.66	1	0	0	0	1	0	5	0.94	C
in15	40	0	0	0.10	0.90	0	0	0	1	0	1	0	0	0	1	0	2	0.85	C
in16	40	0	0.04	0.76	0.20	0	0	0	0.44	0.56	1	0	0	0	1	0	7	0.73	U
in17	20	0	0	0.55	0.45	0	0	0	0.67	0.33	1	0	0	0	1	0	8	0.98	U
in18	40	0	0	0.54	0.46	0	0	0	0.98	0.03	1	0	0	0	1	0	5	1.13	C
in19	40	0	0.43	0.14	0.44	0	0	0	1	0	1	0	0	0	1	0	4	0.73	U
in20	39	0	0	0.18	0.82	0	0	0.38	0.36	0.26	1	0	0	0	1	0	13	1.11	U
in21	40	0	0	0.60	0.40	0	0	0	0.53	0.48	1	0	0	0	1	0	5	0.59	U
in22	42	0	0	0.87	0.13	0	0	0	0.79	0.20	1	0	0	0	1	0	6	1.11	U
in23	40	0	0	0.79	0.21	0	0	0	0.63	0.38	1	0	0	0	1	0	7	0.98	C
in24	36	0	0	0.54	0.46	0	0	0	0.86	0.14	1	0	0	0	1	0	6	0.93	C

in25	40	0	0.05	0.41	0.54	0	0	0	0.81	0.19	1	0	0	0	1	0	8	1.53	0.84	C
in26	40	0	0	0.65	0.35	0	0	0.01	0.61	0.38	1	0	0	0	1	0	9	0.07	1.00	C
Indiana	1002	0	0.04	0.54	0.42	0	0	0.02	0.72	0.27	1	0	0	0	1	0				
io1	40	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1	monomorphic	U	
io2	5	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1	monomorphic,		
Iowa	45	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	n < 15			
mi1	39	0	0	0.81	0.19	0	0	0.03	0.84	0.13	1	0	0	0	1	0	5	0.37	0.72	C
mi2	40	0	0	0.73	0.28	0	0	0	1	0	1	0	0	0	1	0	3	0.16	1.02	C
mi3	39	0	0	0.55	0.45	0	0.18	0	0.82	0	1	0	0	0	1	0	6	4	0.88	U
Michigan	118	0	0	0.70	0.30	0	0.06	0.01	0.89	0.04	1	0	0	0	1	0				
mn1	39	0	0	1	0	0	0	0	0.99	0.01	1	0	0	0	1	0	2	4	1.18	U
mn2	9	0.05	0.05	0.55	0.35	0	0	0	0.50	0.50	1	0	0	0	1	0	6	n < 15		
Minnesota	48	0.01	0.01	0.92	0.07	0	0	0	0.90	0.10	1	0	0	0	1	0				
ne1	24	0	0	1	0	0	0	0	0.48	0.52	0.98	0.02	0	0	1	0	2	2.67	0.18	O
ne2	40	0	0.46	0.54	0	0	0	0	0.96	0.04	1	0	0	0	1	0	13	0.28	0.88	C
ne3	35	0	0.23	0.78	0	0	0	0.21	0.59	0.20	1	0	0	0	0.99	0.01	3	2	0.79	U
Nevada	99	0	0.27	0.73	0	0	0	0.08	0.71	0.21	1	0	0	0	1	0				
on1	5	0	0	1	0	0	0	0	0.70	0.30	1	0	0	0	1	0	2	n < 15		
on2	40	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1	monomorphic	U	
Ontario	45	0	0	1	0	0	0	0	0.97	0.03	1	0	0	0	1	0				
ut1	57	0	0.13	0.80	0.07	0	0.22	0.20	0.48	0.10	1	0	0.07	0	0.93	0	18	0.25	0.72	C
ut2	37	0	0.61	0.39	0	0	0	0	0.89	0.11	1	0	0.01	0	0.99	0	8	0.23	1.13	C
ut3	38	0	0.38	0.63	0	0	0	0.03	0.80	0.17	0.93	0.08	0	0	1	0	12	0.86	0.90	C
ut4	40	0	0	1	0	0	0.03	0	0.98	0	0.98	0.03	0	0	1	0	4	0	1.40	C
Utah	172	0	0.26	0.72	0.02	0	0.08	0.07	0.75	0.09	0.98	0.02	0.03	0	0.97	0				
wi1	35	0	0.09	0.86	0.05	0	0	0.25	0.44	0.31	1	0	0	0	1	0	11	0.12	0.80	C
wi2	26	0	0.15	0.27	0.58	0	0	0	0.87	0.41	1	0	0	0	1	0	6	0.19	0.67	C
wi3	21	0	0	0.79	0.21	0	0	0	0.79	0.21	1	0	0	0	1	0	5	0.14	0.92	C
wi4	36	0	0.01	0.75	0.24	0	0	0	0.92	0.08	1	0	0	0	0.99	0.01	6	0.13	0.92	C
wi5	15	0	0	0.90	0.10	0	0	0	0.93	0.07	1	0	0	0	1	0	3	0.73	0.88	C
Wisconsin	133	0	0.06	0.71	0.23	0	0	0.07	0.76	0.17	1	0	0	0	1	0				
wy1	40	0	0.48	0.53	0	0	0.03	0	0.98	0	0.53	0.48	0	0	1	0	3	2	0.29	O
wy2	40	0	0.03	0.68	0.30	0	0	0.05	0.79	0.16	1	0	0	0.10	0.81	0.09	17	1.14	0.95	C
wy3	40	0	0	0.63	0.38	0	0.83	0	0.18	0	1	0	0	0	1	0	4	2.12	0.59	O
wy4	79	0	0.01	0.63	0.28	0.08	0.03	0.08	0.72	0.18	1	0	0	0.03	0.98	0	27	0.53	1.13	C
Wyoming	199	0	0.11	0.62	0.25	0.03	0.18	0.04	0.67	0.10	0.90	0.10	0	0.03	0.95	0.02				
TOTAL	2070	0.001	0.094	0.645	0.257	0.003	0.039	0.036	0.748	0.177	0.986	0.014	0.002	0.003	0.991	0.003				

Abbreviations: G, no. of genotypes; P(HWE), minus log values of a probability being in H.W. equilibrium; GDR, genotypic diversity ratio; bm, breeding mode (where C = cyclic, O = obligatory parthenogenesis, U = uncertain).

among populations of *D. pulicaria* from eastern North America, but the *F* allele was present in populations from four of the six western states. The *M* allele was similarly dominant in all populations at *MPI*, although three additional alleles were detected rarely at some localities.

The *PGM* and *PGI* loci were considerably more variable, with four alleles detected at each locus (Table 3). *PGI* showed evidence of a regional shift in gene frequency, with the slow allele much more common in western than eastern populations (Fig. 1a). Allele frequencies at the *PGM* locus also showed marked variation among sites, but there was no obvious geographical patterning to this diversity (Fig. 1b).

The hierarchical analyses of the regional gene frequency divergence for the two most polymorphic loci (*PGI*, *PGM*) showed that the bulk of variance in gene frequencies existed among the lakes in a single state or province (Table 4). The additional divergence among populations from different states or provinces and between the central and western groups of populations, although significantly different from 0, was negligible compared to the gene frequency differentiation among local populations.

The unweighted pair-group clustering method in BIOSYS-1 showed that genetic distances among populations from the 12 states and provinces were small (Fig. 2). Although there was little evidence of geographical patterning in lake populations of *D. pulicaria*, those

Fig. 1 Geographical patterning of gene frequencies at (a) *PGI* and (b) *PGM* loci in *Daphnia pulicaria*.

Table 4 Hierarchical analysis of gene frequency diversity in populations of *Daphnia pulicaria* for two polymorphic loci

	DS	d.f.	SR	d.f.	RT	d.f.	DT	d.f.
PGI	0.290*	44	0.023*	35	0.025*	4	0.323*	188
PGM	0.242*	33	0	—	0	—	0.230*	141
Mean	0.268*	77	0.007*	35	0.012*	4	0.282*	321

*Significantly different from 0 at $P = 0.001$.

Abbreviations: DS, differentiation among populations (D) from a single state or province (S); SR, differentiation among states (S) in a region (R); RT, differentiation between regions (R) in total area (T); DT, differentiation among populations (D) in total area (T); d.f., degrees of freedom.

Method from Wright, 1978.

Fig. 2 Unweighted pair-group clustering of genetic distances (Nei, 1978) among lake populations of *Daphnia pulicaria* from 12 states and provinces in North America, based on frequencies of seven commonly variable loci. *Data on *D. pulex* and pond *D. pulicaria* from Hebert *et al.* (1993) were included for comparison.

from pond habitats in western Canada were clearly distinct from the lake populations.

Breeding system diversity

All three parameters employed in breeding system assignments varied considerably among the populations. Clone richness ranged from 2 to 27, with the highest diversity evident in the west. For example, 32 per cent of the populations from western localities contained 10 or more clones, while only 9 per cent of eastern populations exceeded this threshold. Geno-

Fig. 3 Genotypic characteristics of *Daphnia pulicaria* populations from lakes in central and western North America. ■, obligate asexual; ●, cyclic parthenogens; ▲, uncertain. Abbreviations: GDR, genotypic diversity ratio; HWE, H. W. equilibrium.

typic frequencies in most populations approximated H. W. equilibrium, but large deviations were detected at a few sites. Finally, GDRs ranged from a low of 0.18 to a high of 1.40.

The breeding system assignments based on these three parameters suggested that 62 per cent of the populations reproduce by cyclic parthenogenesis, while 17 per cent are obligate asexuals (Fig. 3). Breeding system assignments for the other 21 per cent of the populations were uncertain. Two of the populations included in this 'uncertain' category were completely invariant, making a genetic determination of breeding system impossible. However, the majority of populations in this category were genetically variable, but showed either a lower number of genotypes than expected in a sexual population or significant H. W. deviations.

Three of the Indiana populations were resampled in consecutive years and the Lake Mendota population was sampled in May and July of 1991. Although samples from one of the Indiana lakes (Wawasee) were consistently designated as cyclic parthenogens, populations from the other Indiana lake (Loon) and Lake Mendota were designated as uncertain on one occasion and as cyclically parthenogenetic for the other. Both populations were treated as cyclical parthenogens. The Indiana population at Waubesa, which was sampled twice, showed a major shift in genotypic composition, which led to its categorization as a cyclical parthenogen in 1990 and an obligate parthenogen in 1991. Because of this shift, the population was assigned an uncertain breeding mode (Table 3).

Fig. 4 Breeding system assignments for individual populations of *Daphnia pulicaria*. The numerals beside each pie in Indiana indicate the number of populations assigned to each category.

There was some evidence of a significant geographical shift in breeding systems ($G_{adj} = 5.79$, d.f. = 1, $P < 0.05$) since 36 per cent of the populations (7 of 19) from the western states appeared to reproduce by obligate parthenogenesis compared with only 6 per cent of the central populations (2 of 33). The detailed study of lakes in Indiana provided evidence of the local co-occurrence of both breeding systems since although most of the populations were cyclic parthenogens, two lakes in this state appeared to contain obligately asexual populations (Fig. 4).

Discussion

Studies of breeding system diversity in the *D. pulex* complex have been complicated by difficulties in discriminating between *D. pulex* and *D. pulicaria*. Over the past decade it has become clear that much of this taxonomic confusion arises because of the prevalence of F_1 hybrids between the two species. Recent work has indicated that diagnostic substitutions at *LDH* and *PEP* can be employed in taxon recognition (Hebert *et al.*, 1989a; 1993). The present study is the first to use a genetic criterion in the analysis of lake-dwelling members of the complex. This analysis indicated that *D. pulicaria* dominates lake habitats across North America, but also suggested the presence of *D. pulex* and F_1 hybrids in some lakes. There is a need to rule out the possibility that some populations of *D. pulicaria* are polymorphic at *LDH* due to introgression with *D. pulex*. This possibility does not seem unlikely in light of apparent introgression at other loci and the

pulicaria-like head shape of individuals that were assigned to *D. pulex* on the basis of their *LDH* phenotype. However, in the absence of such information, the present study follows past practice in defining membership in *D. pulicaria* on the basis of homozygosity for the *F* allele at *LDH*. It is important to emphasize that the exclusion of information on any polymorphic locus will not bias final conclusions concerning the breeding system employed by the population under study.

An earlier study (Hebert *et al.*, 1993) involving the genetic analysis of more than 500 populations of the *D. pulex* complex from ponds showed that *D. pulex* was broadly distributed across Canada, while *D. pulicaria* was present only in the west. In the west, *D. pulicaria* was ordinarily dominant in ponds with the largest surface areas. The present study confirms that *D. pulicaria* is the dominant taxon in lakes across much of the continent, a result which might have been anticipated on the basis of prior morphological studies (Brooks, 1957; Brandlová *et al.*, 1972; Hrbáček, 1987).

Populations of *D. pulicaria* in lakes show marked local variation in gene frequency, comparable in extent to that detected in other populations of pond zooplankton (Hebert, 1987; Boileau *et al.*, 1992). In common with these earlier studies, the extent of divergence is only modestly increased as the geographic scope of investigation is extended. Certainly, lake populations of *D. pulicaria* show little geographic patterning in gene frequencies across North America. Genetic distance analysis does suggest, however, that pond populations of *D. pulicaria* show genetic divergence from lake populations. In part, this divergence

appears to reflect the greater exposure of pond *D. pulicaria* to introgression from *D. pulex*. For instance, the *S* allele at *FUM*, which occurs at a frequency of 38 per cent in western populations of *D. pulex*, occurs at a frequency of 3 per cent in pond *D. pulicaria*, but was never detected in lake *D. pulicaria*. Similarly the *F* allele at *GOT*, which has a frequency of 35 per cent in *D. pulex*, was more than twice as common in pond than lake *D. pulicaria* (5 per cent versus 2 per cent).

There is some experimental evidence that supports the possibility of introgression between these taxa. Innes and Hebert (1988) experimentally produced hybrids between *D. pulex* and *D. pulicaria*, while Larsson (1991) carried out a backcross between a natural F_1 hybrid and *D. pulex*. In the second experiment the backcross offspring were not only viable, but also apparently able to reproduce sexually. Both the gene frequency data from natural population surveys and these experimental studies suggest that introgression is likely between these two species. However, their morphological differences and marked genetic divergence support their continued taxonomic recognition.

Prior work (Hebert *et al.*, 1993) has established that populations of *D. pulicaria* from pond habitats in western Canada employ two modes of reproduction — cyclic parthenogenesis and obligate parthenogenesis. Most populations from the prairie provinces are obligate asexuals, while cyclic parthenogens are dominant in British Columbia. This study suggests that lake populations of *D. pulicaria* show the same breeding system diversity. There is a need to verify the occurrence of obligately asexual populations in lakes, as it can be argued that apparent examples simply represent cyclically parthenogenetic populations exposed to little recruitment from sexual eggs. This explanation is perhaps made less likely by the detection in this study of a geographical pattern suggesting the greater abundance of obligate asexuals in the western than central group of populations. However, this hypothesis can only be definitively tested through breeding studies on isofemale lines obtained from lakes dominated by asexual lineages.

While the occurrence of obligate asexuality requires validation, the conclusion that most lake populations of *D. pulicaria* reproduce by cyclic parthenogenesis is robust. It is very unlikely that genotypic distributions will, by chance, approximate those expected in a sexual population. This observation further suggests that populations of *D. pulicaria* are exposed to a substantial amount of sexual recruitment. When coupled with the results of an earlier study (Hebert *et al.*, 1993) the present analysis suggests a divergence in breeding system linked to habitat, with pond populations of *D. pulicaria* dominated by obligate asexuals and lakes by

cyclic parthenogens. Efforts to verify this dichotomy should focus on sites in central Canada, where large numbers of lake populations of *D. pulicaria* occur in proximity to pond populations already known to be dominated by obligate asexuals.

Cyclic parthenogenesis is clearly the ancestral breeding system in the genus *Daphnia* and few species have made the transition to obligate asexuality. It is intriguing that *D. pulex*, *D. pulicaria* and a third closely related taxon (*D. middendorffiana*) are the only species in the subgenus *Daphnia* known to reproduce in this fashion (Hebert & McWalter, 1983; Weider *et al.*, 1987; Hebert *et al.*, 1988; Lynch *et al.*, 1989). Innes and Hebert (1988) have attributed the adoption of asexuality to a sex-linked meiosis suppressor that permits the 'contagious' spread of asexuality via interbreeding. The growing evidence of introgression between these species supports the conclusion that the breeding system transitions in all three species owe their origin to the diffusion of a single mutation across species boundaries.

The parameters determining breeding system distributions are less clear. It is now known that obligately asexual lineages of the *D. pulex* complex are not restricted to North America. There is evidence that populations from mesic sites in Europe largely reproduce by cyclic parthenogenesis (Ruvinsky & Lobkov, 1981; Ward & Geissler, 1988; Hebert *et al.*, 1989b), while those from montane and tundra habitats (Černý, unpub. obs.; Hobaek *et al.*, in press; Ward *et al.*, in prep.) are obligate parthenogens. Detailed studies in North America have shown broad geographical patterning in breeding system shifts. Populations of *D. pulex* show, for example, a shift from obligate to cyclic parthenogenesis which is closely coincident with the prairie-forest ecotone (Hebert *et al.*, 1993). The present study hints at a new level of complexity, suggesting that neighbouring sympatric populations of *D. pulicaria* show differing breeding systems with pond populations dominated by obligate asexuals and lakes by cyclic asexuals. It is becoming increasingly clear that breeding system variation in the *D. pulex* complex is both dynamically and structurally complex. The exploration of such complexity seems likely to contribute to our understanding of the evolutionary fate of asexuals.

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