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Clonal Coexistence in *Daphnia pulex* (Leydig): Another Planktonic Paradox

Abstract. *Allozyme variation is common in populations of* Daphnia pulex *reproducing by obligate parthenogenesis. The genetic diversity within populations results from the coexistence of genetically different clones. Twenty-two clones were recognized in the eleven populations surveyed, of which as many as seven were found in a single habitat.*

A shallow well-mixed lake or pond is typically inhabited by a number of phytoplankton species, all competing for light and nutrients. Hutchinson (*1*) termed this the "paradox of the plankton," pointing out that such coexistence seems to violate the principle of competitive exclusion. Explanations of phytoplankton diversity have emphasized temporal and spatial heterogeneity in the environment (*1*, *2*) as well as the possibility that algal populations are limited by an array of nutrients (*3*). In contrast to the phytoplankton, early studies suggested that coexisting zooplankton species occupied distinct niches. Hutchinson (*4*), for example, pointed out that coexisting copepods generally differ widely in size and suggested that these size differences were correlated with the utilization of different resources. Recent work on cladocerans has clouded the issue. It is now recognized that many of the "species" described by classical systematists are species complexes (*5*). These morphologically similar species not only coexist but also use similar resources, despite the fact that populations are often resource-limited (*6*). The limits to coexistence are not clear. Genetic studies on *Daphnia magna* have shown that large numbers of clones often coexist (*7*). However, as this species is capable of sexual reproduction, it can be argued that clonal coexistence is short term, for new clones may enter the population from sexual eggs.

We now report the results of a study of allozyme variation in natural populations of *Daphnia pulex*, one of the most common zooplankters in pond habitats throughout Eurasia and North America. This species is normally thought to reproduce by cyclical parthenogenesis (*8*, *9*). Species of *Daphnia* reproducing in this fashion produce both parthenogenetic (*10*) and sexual eggs. The sexual eggs are enclosed within a protective structure known as an ephippium and must be fertilized if they are to develop. Some species such as *D. middendorffiana* and *D. cephalata* produce their ephippial eggs parthenogenetically —they are obligate parthenogens. There have been a few reports of populations of *D. pulex* reproducing in this fashion (*11*). We find that such asexual populations are not rare; all of our populations reproduce by obligate parthenogenesis.

We studied nine populations inhabiting ponds in urban or farmland habitats in southwestern Ontario, and two others in natural woodland pools. The habitats were filled with water only during the spring and early summer. The populations were sampled in the early spring shortly after their reestablishment from ephippial eggs.

Specimens for electrophoresis were stored alive at 5°C and used within 4 days of collection. The electrophoretic studies were carried out on single individuals. At least 48 individuals were analyzed from each population for the following enzymes: fumarase (FUM), glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), tetrazolium oxidase (TO), xanthine dehydrogenase (XDH), amylase (AMY), and glutamate oxaloacetate transaminase (GOT) (*12*). Laboratory clones were established from at least 24 randomly chosen parthenogenetic females from each population (*13*). These clones were used to determine associations between genotypes at different loci.

Table 1. Genotypic frequencies at four polymorphic loci in populations of *Daphnia pulex* from southwestern Ontario. Frequencies were determined by electrophoresis of samples from single individuals taken directly from the populations. AMY 2 data are absent because of scoring difficulties.

Population	LDH			PGI				PGM						AMY1					
	N	SS	SF	*N*	SS	SF	FF	*N*	SS	SM	MM	MF	FF	*N*	SS	SM	MM	MF	FF
Cedar Springs	192	.005	.995	278	.126	.126	.748	189	.646		.339	.016		50		.02	.04	.94	
Charing Cross I	72		1.00	134		.746	.254	118	.263		.729			72				1.00	
Charing Cross II	48		1.00	202		.03	.97	174	.983		.017			44				1.00	
Cottam	309	.89	.11	216		.023	.977	201	.06		.264	.677		117				.12	.88
Kingsville	120		1.00	168			1.00	168	.006		.030	.964		141	.028	.035	.936		
Windsor I	222	.356	.644	95			1.00	287	.631		.369			71		.028		.761	.211
Windsor II	191		1.00	120			1.00	217	.350		.650			120		.658		.347	
Windsor III	186	.027	.973	96			1.00	216	.926		.014	.060		167			.042	.946	.012
Bloomfield	96		1.00	96			1.00	96			1.00			72				1.00	
Rondeau I	96	1.00		96		.26	.74	72	.028		.042	.333	.597	72				.014	.986
Rondeau II	72	1.00		48		.75	.25	47	.021	.021	.191	.745	.021	48				.104	.896

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No allozyme variation was detected at six of the loci (FUM, G6PDH, MDH, TO, XDH, GOT), but variation was frequent at the other five (*14*). Two LDH phenotypes were observed; the homozygote pattern had three widely spaced bands (*15*) and the heterozygote pattern had nine bands. Two alleles were present at the PGI locus, and all three expected phenotypes were seen. Three alleles were present at the PGM and AMY 1 loci and at each locus five of the six possible phenotypes were seen (SM heterozygote absent at PGM and SS homozygote missing at AMY 1). There were also three alleles present at the AMY 2 locus, but only three of the six possible phenotypes were seen (missing were SS, FF, SF).

Genotypic frequencies varied markedly among the populations (Table 1). At the LDH locus, some populations contained only the LDH homozygote, while others contained only the LDH heterozygote. Where both LDH genotypes were present, their relative frequencies varied among the individual populations. Genotypic frequencies often deviated markedly from Hardy-Weinberg expectations. Heterozygote excesses were noted at some loci, and deficiencies at others. For instance, all individuals in the Windsor II population were heterozygous at the LDH locus, but heterozygotes were lacking at the PGM locus despite the presence of two different homozygotes.

When clones were scored for their phenotype at the five polymorphic loci, clear associations of genotypes at different loci were detected (Table 2). In the Windsor I population all clones homozygous at the LDH locus were PGM-MM homozygotes, while most of the clones heterozygous at LDH were PGM-SS homozygotes. Similar associations were noted in the other populations. In one population only a single clone could be recognized, whereas in the Rondeau I and Cedar Springs populations seven different clones were present. In most habitats there were only two or three common clones. In the 11 populations, 22 different clones were detected. Certain clones were much more common than others. For example, clone 1 was present in seven of the nine urban and farmland ponds.

Temporal changes in clone frequencies were observed in two populations that were reanalyzed on several occasions. When the Charing Cross population was first analyzed in May 1978, clone 1 made up 94 percent of the population, but in June 1978 its frequency had declined to 25 percent. In 1979 clone frequencies also shifted seasonally, but in the opposite direction to that noted in 1978—clone 1 increased in frequency from 20 percent in April 1979 to 68 percent in June. Changes in clone frequencies were also noted in the Windsor I population. In early April of 1979, clone 13 made up 31 percent of the population, 42 percent in late April, and 27 percent in early May, but it was absent from a sample of 144 individuals taken at the end of May.

Our study has shown that allozyme variation is prevalent in natural populations of *Daphnia pulex*. Yet several lines of evidence indicated that our populations were reproducing by obligate parthenogenesis. Males were absent from populations during periods of ephippia production and, in the laboratory, isolated females released ephippial eggs (*16*). These unfertilized eggs were viable, and individuals hatching from them had the maternal genotype. Genotypic characteristics of the populations were also not compatible with the assumption that ephippial eggs were produced sexually. Prior work on allozyme variation in cyclical parthenogens has shown that genotypic frequencies at single gene loci are usually in good agreement with Hardy-Weinberg expectations in populations that have just been reestablished from ephippial eggs (*17*). The present populations show gross Hardy-Weinberg disturbances as did those studied by Berger and Sutherland (*9*). These deviations cannot be explained by any form of assortative mating because genotypic frequencies at some loci show a heterozygote excess, whereas other loci show a heterozygote deficiency. These large Hardy-Weinberg deviations, plus the observation of

Table 2. Clonal complements of the eleven populations. These data were obtained by electrophoresis of clones isolated from natural populations.

Population	Clone	Frequency	Genotype				
			LDH	PGM	PGI	AMY 1	AMY 2
Cedar Springs	1	37	SF	SS	FF	MF	MM
	2	3	SF	MM	FF	MF	MM
	3	1	SF	MM	SS	MF	MM
	4	1	SF	MM	SS	MF	SM
	5	2	SF	MF	FF	MM	SM
	6	2	SF	MM	SF	MF	SM
	7	1	SF	MF	FF	SM	MM
Charing Cross I	6	33	SF	MM	SF	MF	SM
	1	15	SF	SS	FF	MF	MM
Charing Cross II	1	23	SF	SS	FF	MF	MM
	6	1	SF	MM	SF	MF	SM
Cottam	8	41	SS	MF	FF	FF	MM
	9	34	SS	MM	FF	FF	MM
	1	5	SF	SS	FF	MF	MM
	6	5	SF	MM	SF	MF	SM
	10	11	SS	MF	FF	FF	MF
Kingsville	11	94	SF	MF	FF	MM	MF
	12	2	SF	MM	FF	SM	MM
Windsor I	1	39	SF	SS	FF	MF	MM
	13	6	SS	MM	FF	FF	Null
	12	2	SF	MM	FF	SM	MM
Windsor II	12	58	SF	MM	FF	SM	MM
	1	38	SF	SS	FF	MF	MM
Windsor III	1	68	SF	SS	FF	MF	MM
	11	3	SF	MF	FF	MM	MF
	14	1	SF	SS	FF	MM	MF
Bloomfield	6	48	SF	MM	SF	MF	SM
Rondeau I	15	27	SS	FF	FF	FF	Null
	8	14	SS	MF	FF	FF	MM
	16	2	SS	MM	SF	FF	MM
	17	2	SS	SS	FF	FF	MM
	18	1	SS	FF	SF	FF	MM
	19	1	SS	MF	SF	FF	MM
	20	1	SS	MF	FF	MF	MM
Rondeau II	19	13	SS	MF	SF	FF	MM
	8	7	SS	MF	FF	FF	MM
	17	1	SS	SS	FF	FF	MM
	21	1	SS	SM	FF	MF	MM
	16	1	SS	MM	SF	FF	MM
	22	1	SS	FF	FF	FF	MM

marked multilocus disequilibrium, support the view that the populations of *D. pulex* were not capable of sexual reproduction.

The demonstration that *D. pulex* populations consist of genetically different clones raises significant evolutionary and ecological problems. The nonoccurrence of competitive exclusion suggests that the relative fitnesses of clones are unstable. The situation seems to be one of contemporaneous disequilibrium, in which environmental changes shift genotypic fitnesses before exclusion results. The coexistence of clones needs to be considered in relation to current views on the limits to niche overlap. Studies on vertebrate communities have suggested that there are constraints on the amount of resource overlap allowable between coexisting species (*18*). There is growing evidence that zooplankton communities fail to show such restraints on resource overlap—the clones of *D. pulex* probably use identical food items. This tolerance of overlap does not result from the presence of unlimited resources; the growth of zooplankton populations is normally limited by food shortage. A more fundamental reason for the tolerance of resource overlap can be offered. Stable species or clonal coexistence depends upon equality of relative fitnesses—although this equality may exist only as a long-term average. It is important to recognize that the limiting resource is not, in many cases, the primary determinant of relative fitness. For instance, a *Daphnia* population could be food-limited, but fitness differences among genotypes in the population be caused by differing susceptibility to some physical factor, such as oxygen tension. Only in cases where relative fitnesses are determined primarily by the limiting resource should there be any limit to resource overlap. It seems likely that studies on interactions between coexisting clones will contribute much to our understanding of constraints on species packing.

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13. Individual clones were maintained in 100-ml beakers containing synthetic pond water made by adding 48 mg of $NaHCO_3$, 38 mg of $CaSO_4 \cdot 2H_2O$, 30 mg of $MgSO_4$, and 0.5 mg of KCl to 1 liter of distilled H_2O. Animals were fed every other day with aquarium-cultured algae (primarily *Scenedesmus*). Mortality during the establishment of clones was usually less than 5 percent.
14. The variation was shown to be heritable by isolating individual females and typing their parthenogenetic progeny. In all cases maternal and offspring phenotypes corresponded. Interpreting the genetic basis of the allozyme variation was complicated because of the absence of sexual reproduction. Variation at the LDH, PGI, and PGM loci was interpreted by comparing *D. pulex* phenotypes with those observed in sexually reproducing species such as *D. magna* and *D. carinata*. Studies on these species have shown that PGI and PGM homozygotes have a single banded phenotype, while PGM heterozygotes have two bands (PGM is a monomer) and PGI heterozygotes have three bands (PGI is a dimer). One *D. pulex* LDH phenotype was similar to that observed in homozygous individuals of sexually reproducing species. The second LDH phenotype had nine bands, spaced in three zones. In each zone the central band stained most strongly. Individuals possessing the phenotype were presumably heterozygous at the LDH locus. If so, the functional LDH molecule is a dimer and all three zones of LDH activity in the usual homozygote are synthesized by a single locus. Amylase phenotypes were not analyzed in *D. magna* or *D. carinata*, but the patterns seen in *D. pulex* are compatible with the hypothesis that AMY homozygotes have a single banded phenotype, while heterozygotes have two.
15. More than three bands may be present in *Daphnia* homozygous at the LDH locus as a result of proteolytic degradation of LDH molecules. Such breakdown was occasionally seen in *D. pulex*, but could be readily distinguished from the heterozygote pattern.
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Elastase Digestion of Demembranated Sperm Flagella

Abstract. *The changes in adenosine triphosphate-reactivated motility resulting from digestion of Triton-demembranated sea urchin sperm flagella by elastase are those expected if the elastic interdoublet linkages between flagellar microtubules are particularly sensitive to digestion by elastase and take part in regulating the amplitude of flagellar bending.*

The nine microtubular doublets in the periphery of flagellar axonemes are held together at 96-nm intervals by "interdoublet linkages" (*1*), which appear to consist, at least in part, of a protein that has been named "nexin" (*2*). These interdoublet linkages must be highly extensible to accommodate the sliding between microtubules that occurs during flagellar bending. Extension to between two to four times the rest length appears to be required during normal bending. Even greater extension of the interdoublet linkages has been seen in electron micrographs of partially disintegrated sperm flagella (*3*).

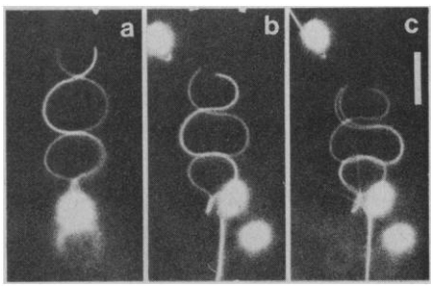

Fig. 1. Multiple-flash dark-field photomicrographs of a motile, demembranated sea urchin spermatozoon during exposure to elastase. Photographs were taken (a) 47 seconds, (b) 192 seconds, and (c) 266 seconds after mixing with elastase; this flagellum disintegrated at 306 seconds. Each photograph was taken with three flashes, with the flash frequency adjusted to twice the beat frequency. Superposition of the first and third images then verifies the accuracy of the adjustment of flash frequency and the recorded beat frequency (scale bar, 10 μm).

Active sliding can occur over distances much greater than those normally encountered during flagellar bending if sperm flagella are first digested with trypsin (*4*). Trypsin digestion causes damage to both the interdoublet linkages and the radial spokes, which can be detected by electron microscopy (*5*). The interdoublet linkages are more likely to be the structures that restrict the range of sliding since *Chlamydomonas* mutants lacking radial spokes still require trypsin digestion before active sliding disintegration can occur (*6*).

If the elastic resistance of these inter-

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