Ribosomal DNA Variation in *Daphnia pulex*[1]

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Variation in the ribosomal DNA (rDNA) gene family was surveyed in five cyclically parthenogenetic populations of *Daphnia pulex* from central Illinois and in obligately parthenogenetic clones from Illinois and Ontario. A total of 37 distinct rDNA repeat types were identified on the basis of restriction-site and repeat-length polymorphism in a sample of 90 isolates. Repeat-type diversity was high within cyclic populations; however, individuals possessed only a small subset of the repeat-type variation present in each population. The distribution of repeat types within and among individuals suggested that new variants spread within rDNA arrays much faster than arrays carrying the variants spread within populations. This observation is contrary to a model developed by Ohta and Dover for strictly sexual organisms. Previous surveys of isozyme and mitochondrial DNA variation in these populations showed that gene flow among them is limited. Hierarchical analysis of rDNA restriction-site variation was consistent with this observation and showed that genetic divergence accumulates between populations for rDNA almost as rapidly as it does for single-copy nuclear genes (isozymes). Analysis of rDNA variation in obligately parthenogenetic clones provided evidence that both intra- and interchromosomal exchanges occur between rDNA arrays in the absence of meiosis. Moreover, individuals reproducing by obligate parthenogenesis possessed fewer rDNA repeat types, on average, than did individuals from cyclic populations, suggesting that there is a net loss of rDNA repeat-type variability within obligately clonal lineages over time. Preliminary analyses of two additional species, *D. pulicaria* and *D. obtusa,* revealed several restriction-site polymorphisms that were found in more than one of the three species. The existence of such shared polymorphisms advises caution in the use of multigene-family variation to infer phylogenies among species when levels of intraspecific variation have not been assessed.

Introduction

A common feature of eukaryotic genomes is the occurrence of multigene families. The individual members of such families do not evolve independently of one another, a phenomenon known as concerted evolution (Arnheim 1983). Genetic interactions at the level of the DNA molecule itself have been invoked to account for the ability of genes within a multigene family to evolve in concert (Dover 1982). Such interactions include unequal crossing-over, gene conversion, and transposition.

The spread of a new mutational variant throughout a gene family and throughout a population is a function of the rate of DNA exchange at the molecular level and of genetic drift, migration, and natural selection at the population level. Dover (1982) has termed the process whereby a new variant becomes fixed within a population in the absence of natural selection "molecular drive." As a consequence of this process,

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relative homogeneity of multigene families can be maintained within species despite their divergence between species. The distribution and level of multigene-family variation within species will depend on a variety of factors including the size of the gene family, the number of chromosomes on which it occurs, the rate of DNA exchange at the molecular level, and population structure (size, migration rate, and breeding system).

Ribosomal DNA (rDNA) is a multigene family in which structure and function have been well characterized (reviewed in Long and Dawid 1980; Gerbi 1985). In eukaryotes, rDNA is composed of tandem arrays of a basic repeat consisting of a transcription unit and an intergenic spacer (IGS). In most species studied to date, the IGS of each repeat contains a tandem array of small "subrepeats" which are thought to be involved in the control of rRNA transcription (Gerbi 1985; Rogers and Bendich 1987*b*, and references within).

Because of its universal presence, its relatively simple structure, and the fact that different regions of its basic repeat unit evolve at different rates, rDNA has attracted attention as a phylogenetic marker. Since it appears to evolve in a concerted fashion, it is often assumed that the rDNA of a single individual is representative of the entire species in phylogenetic studies (Hasegawa et al. 1985; Hillis and Davis 1986; Verma and Dutta 1987; Field et al. 1988; Mindell and Honeycutt 1989; Vossbrinck and Friedman 1989). However, it is now clear that variation does exist within and between populations of the same species. Much of the work done to date has focused on the distribution of repeat-length variation caused by changes in the number of IGS subrepeats (Saghai-Maroof et al. 1984; Flavell et al. 1986; Rogers et al. 1986; Learn and Schaal 1987; Williams et al. 1987). These changes are thought to be the result of unequal crossing-over between subrepeats (Coen et al. 1982) and are thus a product of the homogenization process itself. Those studies that have sought sequence variation within populations, either directly or with the use of restriction endonucleases, have found that it does occur (Passananti et al. 1983; Schäfer and Kunz 1985; Sytsma and Schaal 1985; Suzuki et al. 1986, 1987; Bousquet et al. 1989; Hintz et al. 1989; King and Schaal 1989; McLain et al. 1989; Molnar and Fedak 1989; Sites and Davis 1989). However, the number of individuals sampled and/or the proportion of the rDNA repeat surveyed has often been quite small.

The purpose of the present study is to examine population-level variation in the rDNA of the freshwater cladoceran *Daphnia pulex*. There are two modes of reproduction in this species: cyclic and obligate parthenogenesis. During the spring and summer, females produce eggs via ameiotic parthenogenesis. When environmental conditions deteriorate, males and diapausing or "ephippial" eggs are produced. The ephippial eggs of the cyclic parthenogens are produced meiotically and require fertilization. The obligate parthenogens produce their ephippial eggs ameiotically, so they are strictly clonal. In the temporary ponds surveyed in the present study, the *Daphnia* populations must produce resting eggs at least once each year. The existence of both types of reproduction in this species provides a unique opportunity to study the involvement of meiosis in multigene-family evolution.

Isozyme and mitochondrial DNA (mtDNA) surveys of *D. pulex* in Illinois, Michigan, and Ontario have shown that populations of the cyclic parthenogen are highly differentiated, presumably as a result of limited dispersal ability and fluctuating selection on isozyme loci (Lynch 1987; Hebert et al. 1989; Crease et al. 1989, 1990). On the basis of isozyme data, the obligate parthenogens have been divided into two groups: "forest" clones and "urban" clones (Hebert and Crease 1980, 1983). Forest clones are thought to be derived from cyclic *D. pulex* via the spread of a sex-limited

meiosis suppressor (Hebert 1981). The urban clones are thought to be F_1 hybrids between *D. pulex* and a closely related species, *D. pulicaria* (Hebert et al. 1989). Surveys of mtDNA variation in conjunction with isozyme data are consistent with these ideas. These surveys also show that both clonal groups are polyphyletic and have an evolutionarily recent origin from their cyclic ancestors (Crease et al. 1989, 1990; Hebert et al. 1989).

In the present study, *D. pulex* reproducing by cyclic and obligate parthenogenesis were surveyed for rDNA variation by using restriction-endonuclease analysis in order to address the following questions: First, what is the level of rDNA variation within and between cyclically parthenogenetic populations? Second, what effect does the loss of sexual reproduction have on the pattern of rDNA variation in obligately parthenogenetic clones? The present study, in combination with the work of Crease et al. (1990), will provide the opportunity to compare, in the same group of populations, patterns of genetic divergence at mitochondrial, single-copy nuclear (isozyme), and multigene-family loci.

Material and Methods

Daphnia Samples

Ponds were sampled in the spring, soon after the *Daphnia* populations were reestablished from ephippial eggs. Animals were maintained in culture in the lab parthenogenetically until sufficient numbers were available for DNA extraction.

Five populations of cyclic parthenogens, designated BU, KA, PA, SA, and SD, from central Illinois and Indiana were chosen for analysis. Their locations are shown in figure 4. Populations prefixed with S (SA and SD) are located on the Smith Farm (SF; see fig. 4). These five populations have been characterized, with respect to both mtDNA and isozyme variation, by Crease et al. (1990). Also included in the present study were 10 isolates from a population of *D. pulicaria* in Lake Mendota near Madison, Wisc., and one *D. pulicaria* isolate each from Miner Lake, near Kingston, Ont., and Peche Island, in the Detroit River. A single isolate of *D. obtusa* from BU was also analyzed.

Twenty-three isolates of *D. pulex* reproducing by obligate parthenogenesis were assayed. Twelve of these were identified in an isozyme-and-mtDNA survey of *D. pulex* by Crease et al. (1990). They represent one isolate of each genotype from each of eight populations, designated BU, DW, LT, TR, TS, SB, SP, and SW, in Illinois. The locations of these populations are shown in figure 4. Populations prefixed with S (SB, SP, and SW) are located on SF. BU is a mixed population containing both cyclic parthenogens (see above) and an obligately parthenogenetic clone. The genotype designations of the isolates correspond to those in Crease et al. (1990)—1.5(TS), 2.4(LT), 2.6(BU), 3.2(LT), 3.2(SB), 3.2(SW), 4.1(DW), 4.1(LT), 4.1(SP), 4.1(TR), 5.3(SP), and 6.7(TR), where the first number refers to the isozyme genotype, the second number refers to the mitochondrial genotype, and the population from which the isolate came is given in parentheses. All of these isolates are urban clones, except for 6.7(TR), which is a forest clone. The other 11 isolates are forest clones identified in an isozyme-and-mtDNA survey of *D. pulex* in Essex County, Ont. (Crease et al. 1989; Hebert et al. 1989). Their genotype designations correspond to those in Crease et al. (1989): 13.28, 16.9, 18.6, 21.31, 22.20, 22.30, 24.14, 25.19, 30.4, 35.34, and 36.35, where the first number refers to the isozyme genotype, and the second number refers to the mitochondrial genotype.

Restriction-Site Analysis

DNA was extracted from each *Daphnia* isolate as described by Crease (1986). A preliminary restriction map of one *Daphnia* isolate was constructed using single and double digests of the following type II restriction endonucleases: *Apa*I, *Bam*HI, *Bcl* I, *Bgl* I, *Bgl* II, *Bst*EII, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Mlu*I, *Nco*I, *Pst*I, *Pvu*II, *Sac*I, *Sal* I, *Stu*I, *Xba*I, and *Xho*I. *Hpa*I and *Kpn*I were also tried and found not to cut the rDNA repeat. All of these enzymes recognize a single 6-bp sequence. Digestion, electrophoresis, blotting, and probing of Southern blots were carried out as described by Crease et al. (1989). A mixture of phage lambda DNA cut singly with *Hin*dIII, *Pvu*I, and *Sac*I was used as a molecular-weight marker on the agarose gels. Fragment sizes were calculated using the algorithm of Schaffer and Sederoff (1981).

Two plasmids containing cloned vertebrate rDNA were used as hybridization probes for the preliminary restriction map. One of these plasmids, I-19, was obtained from Norman Arnheim and contains the mouse 28S gene. The other plasmid, FH84, was obtained from Elizabeth Zimmer and contains the human 18S gene. Probes were labeled with 32P-dCTP by using the random labeling technique of Feinberg and Vogelstein (1983).

To obtain *Daphnia* probes for regions of the rDNA repeat that are not covered by the vertebrate probes, a phage library of one *D. pulex* isolate from PA was constructed. Total genomic *Daphnia* DNA was partially digested with *Bam*HI and then cloned into the phage vector EMBL3 (Promega) by using standard techniques (Maniatis et al. 1982, pp. 282–294). The resulting library was screened with I-19. Most of the clones contained a single *Bam*HI fragment that spans one rDNA repeat minus ~2.5 kb from the middle of the 28S gene (fig. 1). Two fragments were subcloned from one of these recombinant phage into the phagemid vector Bluescript KS M13+ (Stratagene): (1) a 4.0-kb *Eco*RI fragment containing the *D. pulex* 18S gene and part of the ITS and (2) a 5.0-kb *Xho*I fragment containing ~85% of the IGS and 200 bp of the 18S gene (fig. 1). These two subclones were designated DP18S and DPIGS, respectively. Restriction-site variability in the rDNA of the remaining *Daphnia* isolates was assayed using the 20 restriction enzymes listed above and using I-19, DP18S, and DPIGS as hybridization probes.

To examine intraclonal changes in relative repeat-type frequencies, we digested DNA from 10 additional isolates of urban clone 1.5 from population TS by using the enzyme *Bst*EII and then probed it with I-19. This combination allowed the resolution of a site difference between *D. pulex* and *D. pulicaria* as well as length variation in the IGS.

Analysis of rDNA Diversity in Cyclic Parthenogens

Sequence divergence between pairs of repeat types was calculated by the maximum likelihood method of Nei and Tajima (1983). Only restriction-site data were used for this analysis; length variation was ignored. The average number of substitutions per nucleotide site between random pairs of repeat types (hereafter referred to as rDNA variation) within and between individuals and populations was calculated by the method of Lynch and Crease (1990). Individual *Daphnia* often had multiple repeat types, and, as it was not possible to quantify the relative amounts of each, all repeat types within an individual were assumed to be of equal frequency for this analysis. As a consequence of this procedure, rDNA variation within individuals and populations will be overestimated because heterozygosity will be maximized. Conversely, between-

individual and -population rDNA variation will be underestimated because repeat-type frequency differences will be reduced.

The copy number of rDNA repeats per haploid genome in *Artemia* (brine shrimp) has been estimated to be ~320 (Roberts and Vaughn 1982). Preliminary experiments with *D. pulex* suggest that rDNA repeat copy number is on the order of 400–500 (B. Sullender, personal communication). For the purposes of the rDNA analysis, individuals were assigned an rDNA repeat copy number of 420. This number was chosen so that values of all repeat types within individuals would be whole numbers.

The rDNA variation was subdivided into four hierarchical levels (Crease et al. 1990): (1) within individuals, (2) between individuals within populations (ponds), (3) between populations within locales (populations located <1 km from one another), and (4) between locales. In the present study there were four locales, PA, KA, BU, and SF. Two populations from SF (SA and SD) form the basis of the within-locale analysis. One population was sampled from each of the other three locales. The degree of genetic differentiation at each hierarchical level was calculated using the fixation index, *N*, of Lynch and Crease (1990). This *N*-statistic is analogous to Wright's *F*-statistic in that it measures the amount of genetic variation within a megapopulation that is attributable to genetic differentiation among its subpopulations (Hartl 1980). However, in addition to differences among allele frequencies, *N* also explicitly accounts for variation between-alleles at the nucleotide level. Subscripts for *N* are I, for individuals; S, for populations; L, for locales; and R, for the region.

To compare the results of this analysis with previous work on genetic differentiation among *D. pulex* populations at isozyme loci, we will consider the results that Crease et al. (1990) obtained by using the gene diversity index *G'*. *G'*, which is very similar to Nei's gene diversity index *G* (Nei 1986), is calculated in the same way as *N*, except that alleles are not weighted by their genetic distances (Lynch and Crease (1990). Lynch and Crease (1990) have shown that estimates of *N* and *G'* for the same set of data are highly correlated.

Results

Restriction-Map Variation in the rDNA of *Daphnia pulex*

Of the 73 restriction sites mapped, 23 were polymorphic (fig. 1). Most of the polymorphic sites were found in the IGS, as in other studies of intraspecific rDNA variation (Passananti et al. 1983; Schäfer and Kunz 1985; Sytsma and Schaal 1985; Suzuki et al. 1987; Hintz et al. 1989; King and Schaal 1989; McLain and Collins 1989; Sites and Davis 1989). However, there was one polymorphic site in the 28S coding region. Though rare, coding-region polymorphism within species has been observed in other organisms (King and Schaal 1989; Sites and Davis 1989).

The standard length of the rDNA repeat was 13.4 kb. Length variants resulting from both insertions and deletions were also found. Most of these variants mapped to an IGS region which preliminary sequencing studies have shown to consist of tandem subrepeats (T. J. Crease, unpublished data). Thus, the length variation observed was most likely due to changes in the number of these subrepeats, which were either 330 or 200 bp in length (fig. 2). The additional 130-bp insert in the longer subrepeats contained a duplication of 41 bp present in the basic subrepeat. A simple mechanism that could account for the loss of this insert is intrastrand exchange (Walsh 1987).

One length variant in *D. pulex* did not map to the IGS subrepeat region. An insert of 750 bp containing four restriction sites was found between the 3' end of the 28S gene and the IGS subrepeat array (fig. 1). The nature of the insert is unknown

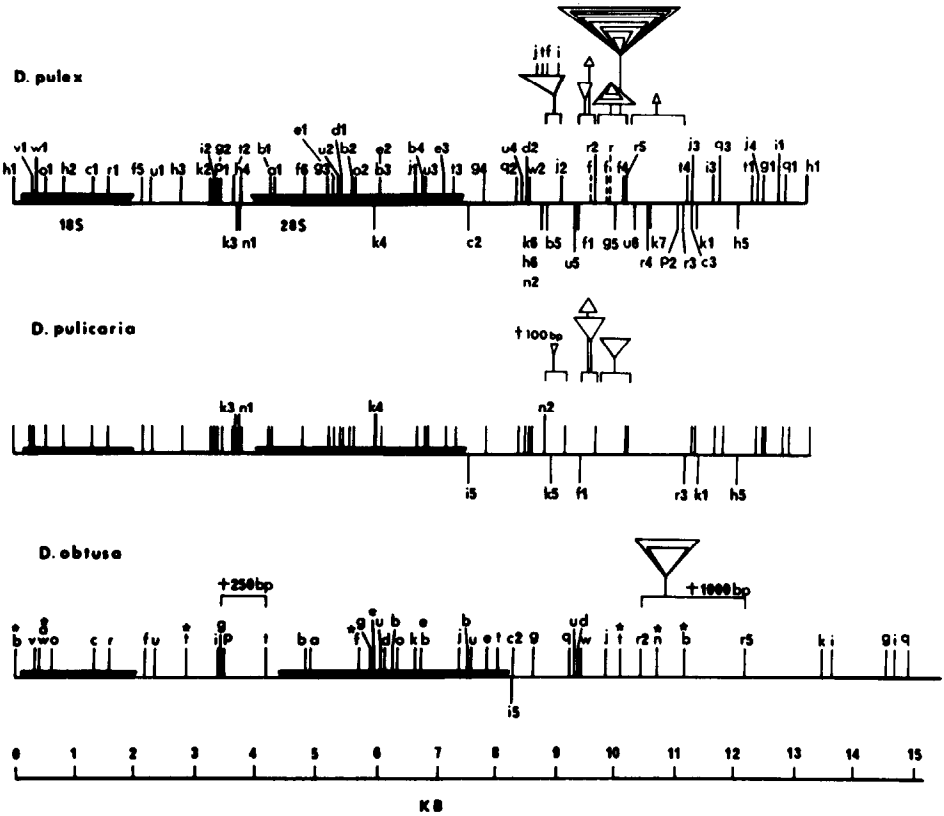

FIG. 1.—Restriction-site maps of the rDNA of *Daphnia pulex, D. pulicaria,* and *D. obtusa.* Restriction sites recognized by the same restriction enzyme are arbitrarily numbered for ease of reference in *D. pulex* and *D. pulicaria.* The approximate location of the 18S and 28S coding regions are shown by the solid boxes. The 5′ end of the 18S gene was determined by sequencing the region upstream from site w1 and comparing it with the *Artemia* 18S gene sequence (Nelles et al. 1984). Conserved restriction sites are shown above the line, and polymorphic sites are shown below the line. The broken vertical lines refer to restriction sites that were not detected by Southern blot analysis but have been detected by direct DNA sequence analysis. Insertions and deletions are indicated, approximately to scale, by triangles pointing toward or away from the map, respectively. Unlabeled sites on the *D. pulicaria* map correspond to sites in *D. pulex.* The three largest triangles on the *D. pulicaria* map represent the average size of several length variants. The brackets over the *D. obtusa* map mark regions where the standard *D. obtusa* repeat differs in length from the standard *D. pulex* repeat. Sites marked with a star are unique to *D. obtusa. Dra*I was not mapped in this isolate. Letter codes for the restriction enzymes are as follows: a = *Apa*I; b = *Bam*HI; c = *Bcl*I; d = *Bgl*I; e = *Bgl*II; f = *Bst*EII; g = *Cla*I; h = *Dra*I; i = *Eco*RI; j = *Eco*RV; k = *Hin*dIII; n = *Mlu*I; o = *Nco*I; p = *Pst*I; q = *Pvu*II; r = *Sac*I; t = *Sal* I; u = *Stu*I; v = *Xba*I; w = *Xho*I.

at present, but its unique restriction-site profile suggests that it is not similar to any other region of the IGS.

Among the entire *D. pulex* sample, a total of 37 rDNA repeat types were identified on the basis of restriction-site and length variation. Genotype descriptions for the repeat types are available from the first author on request. Individual *Daphnia* isolates were often polymorphic for more than one restriction site or length variant and were, therefore, carrying more than one repeat type. For example, as many as four repeat types (−−, +−, −+, and ++, where + indicates the presence of a site and − indicates its absence) could be present in an isolate with two restriction-site polymorphisms. In most cases, appropriate double digests indicated which of the four possibilities were

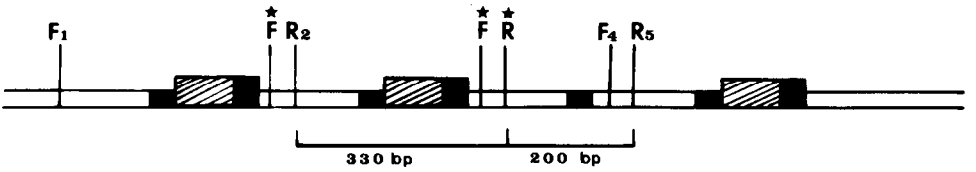

FIG. 2.—Diagram of subrepeat region of IGS of standard 13.4-kb rDNA repeat in *Daphnia pulex*. This figure corresponds to the sequence between sites j2 and t4 in fig. 1. The thicker regions of the line represent the 130-bp insert. The locations of the 41-bp direct repeats are indicated by the solid areas. The diagonally hatched areas indicate sequences unique to the insert. Letter codes for restriction enzymes are as in fig. 1. Sites marked by a star could not be detected by Southern blotting but were detected by sequencing.

actually present. However, in some cases, particularly individuals that were polymorphic for three or more variants, it was not possible to rule out with certainty the presence of some of the many possible repeat types. In such cases, the strategy of Clark (1990) was used to assign repeat-type complements to highly polymorphic isolates. Thus, these repeat types represent the minimum number observed in the sample.

The common repeat types 1, 2, 3, and 4 (table 1) represent the four gametic types (−−, +−, −+, and ++, respectively) for polymorphic sites h5 and k1 (fig. 3). Most of the other repeat types were simple derivatives of these four, either differing in length (repeat types 5–15) or by the presence/absence of one or two polymorphic restriction sites (repeat types 16–26). In most cases, a restriction-site variant was associated with only one of repeat types 1–4. However, the loss of site k3 was associated with three of them (repeat types 22–24), and the gain of site h6 was associated with all four (repeat types 16–19) (fig. 3). If the probability of parallel restriction-site gains and losses is small, the only way to generate all the possible gametic types is through recombination (Schaeffer et al. 1987). That recombination has played an important role is reflected by the fact that many groups of repeat types form closed boxes (fig. 3). Recombination between a pair of repeat types across one diagonal of a box will generate the two repeat types across the other diagonal.

Two groups of repeat types were quite divergent from the four basic types. Repeat types 30–36 differed from these four types at four restriction sites ($k4^-$, $g5^+$, $r4^+$, and $p2^+$), in addition to having long (>1-kb) inserts in the subrepeat region. Repeat types 27–29 also differed from the basic four types at four restriction sites ($k6^+$, $h6^+$, $n2^-$, and $u6^+$), in addition to having the 750-bp insert. The site variants characterizing these two groups were usually not found in repeat types outside the groups.

As based on restriction site variation, mean ± SE sequence divergence between pairs of repeat types was 0.0069 ± 0.0002 with a range of 0.0014–0.0201. The four sites located in the 750-bp insert were omitted from this analysis. Crease et al. (1990) have surveyed restriction-site variation in mtDNA from cyclically parthenogenetic *D. pulex* populations from central Illinois, including the five in the present study. Mean ± SE sequence divergence among mtDNA genotypes from these populations averaged 0.0073 ± 0.0017.

Restriction-Map Variation in the rDNA of *D. pulicaria*

The restriction map of rDNA from *D. pulicaria* was very similar to that of *D. pulex* (fig. 1). Only two sites, i5 and k5, were unique to this species. Conversely, several polymorphic *D. pulex* sites (b5, c2, c3, g5, h6, k6, k7, p2, u5, and u6) were not observed in any of the *D. pulicaria* isolates. The two most common repeat types found in the *D. pulicaria* isolates were $f1^-$-$h5^+$-$k5^+$-$r3^+$ and $f1^-$-$h5^-$-$k5^+$-$r3^+$. Sites

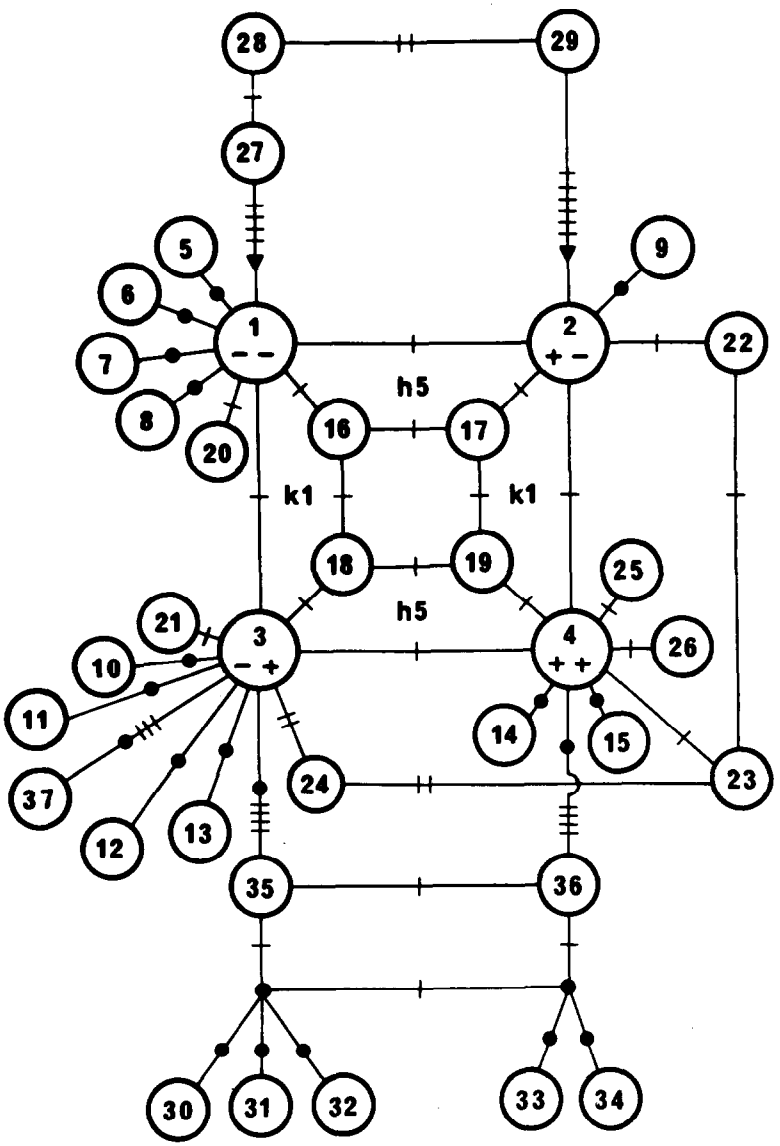

FIG. 3.—Network showing relationship among all *Daphnia pulex* rDNA repeat types. The number of restriction-site differences between repeat types is indicated by the number of hatch marks across the branches. A solid circle indicates a difference in the number of IGS subrepeats. A solid triangle indicates the presence/absence of the 750-bp insert. The presence/absence (+/−) of h5 and k1, respectively, is shown for repeat types 1–4.

k5 and r3 are shown as polymorphic in figure 1, but only two isolates from Lake Mendota and the isolate from Peche Island had repeats lacking these two sites.

Length variation was common among the *D. pulicaria* isolates, with some having as many as three length variants in addition to the standard repeat of 13.5 kb. The additional 100 bp relative to *D. pulex* was due to an insertion between sites n2 and j2, near the 3′ end of the 28S gene (fig. 1). Except for this insert, all other length variation mapped to the region of the subrepeats.

Restriction-Map Variation in the rDNA of *D. obtusa*

The restriction map of rDNA from the *D. obtusa* isolate from BU was similar to that of *D. pulex* in the coding regions (fig. 1). However, both an *Apa*I site in the 18S gene and a *Bst*EII site in the 28S gene of *D. obtusa* were absent in *D. pulex*. Conversely, site f6 in the 28S gene of *D. pulex* was absent in *D. obtusa*. In addition, three sites in the 5.8S/internal spacer region of *D. pulex* were absent in *D. obtusa*. The IGS of each species differed considerably. However, the *D. obtusa* isolate was polymorphic for i5, which was also polymorphic in *D. pulicaria* but not in *D. pulex*, and was fixed for site c2, which was polymorphic in the urban clone 1.5(TS).

The most prevalent repeat type in the *D. obtusa* isolate was ~1,250 bp longer than the standard *D. pulex* repeat, because of insertions in both the 5.8S/internal spacer region and the region between r2 and r5 in the IGS (fig. 1). At present, it is not known whether the region between r2 and r5 is composed of subrepeats. However, length variation was mapped to this region, and the fragment r2–r5 was not visible on Southern blots, suggesting that there are additional *Sac*I sites between r2 and r5.

Hierarchical Analysis of rDNA Variation in *D. pulex* Populations Reproducing by Cyclic Parthenogenesis

Repeat types 1–4 were found in all five populations reproducing by cyclic parthenogenesis (table 1 and fig. 4). However, the other 29 repeat types found in cyclic populations were very rare. One was found in three populations, one was found in two populations, and 27 were found in single populations. Nineteen repeat types were found in only single cyclic individuals. With such a high level of diversity, there is no doubt that many repeat types were not sampled in this survey.

The number of repeat types per cyclic population varied from five in SD to 17 in SA (table 2), with a mean ± SE of 10.4 ± 2.1. Within populations, the average number of repeat types per individual varied from 2.2 in SA to 4.0 in BU (table 2) at the level of resolution of the technique used. Thus, individuals seem to possess only a small subset of the variants present in the population as a whole.

All but one of the 67 cyclic isolates had at least one of repeat types 1–4, and 15 isolates had three or four of them. On the basis of visual inspection of the autoradiographs, it was clear that at least half of the rDNA repeats within any individual were one or more of these four types (except in one individual). On the other hand, the fact that a repeat type was only found in one or a few individuals did not necessarily mean that it was also rare within those individuals. In some cases, the "rare" repeat type appeared to account for ≤40% of the rDNA repeats within an individual.

Estimates of rDNA variation (average number of substitutions per nucleotide site between random pairs of repeat types) within and between individuals were calculated for each cyclic population (table 3). Values of N_{IS} ranged from 0.21 in SD to 0.59 in SA, with a mean ± SE of 0.34 ± 0.11 (table 4). Differentiation at the local and regional levels was lower than that at the population level (N_{SL} = 0.13 ± 0.00 and N_{LR} = 0.06 ± 0.05; table 4). However, these estimates represent the lower bound of differentiation, because of both the overestimation of within-population variation and the underestimation of between-population variation.

Similar hierarchical analyses of isozyme and mtDNA differentiation have been done for cyclically parthenogenetic *D. pulex* populations from central Illinois, including the populations surveyed in the present study (Crease et al. 1990). Estimates of isozyme diversity for these populations were 0.06 ± 0.02 and 0.24 ± 0.09 at the local (G'_{SL}) and regional (G'_{LR}) levels, respectively (Crease et al. 1990). Estimates of mtDNA divergence for these same populations were 0.20 ± 0.11 (N_{SL}) and 0.72 ± 0.17 (N_{LR}).

Table 1
Distribution of rDNA Repeat Types among Populations of *Daphnia pulex* Reproducing by Cyclic Parthenogenesis

	No. in Population				
Repeat Type	KA	BU	PA	SD	SA
1	14	2	4	3	13
2	5	1	3	4	3
3	3	7	3	11	6
4	5	4	11	10	8
5	...	...	...	...	2
6[a]	...	...	...	...	...
7	1	...	...	...	...
8	1	...	...	...	...
9	...	...	...	...	1
10	...	...	...	...	1
11[a]	...	...	...	...	...
12	...	...	...	...	1
13	1	...	...	...	...
14	...	...	...	...	1
15	...	...	...	...	1
16	...	3	1	...	1
17	...	2	...	...	...
18	...	5	...	...	1
19	...	4	...	...	...
20[a]	...	...	...	...	...
21	...	...	...	...	1
22	...	...	1	...	...
23	...	...	1	...	...
24	1	...	...	...	...
25	...	...	...	...	3
26	...	...	2	...	...
27	...	...	...	...	1
28[a]	...	...	...	...	...
29	...	...	6	...	...
30	3	...	...	...	...
31	1	...	...	...	...
32	1	...	...	...	...
33	1	...	...	...	...
34	1	...	...	...	...
35	...	...	...	3	...
36	...	...	...	...	1
37	...	...	...	...	1

[a] Found only in the obligate parthenogens (see tables 5 and 6).

rDNA Variation in the Obligate Parthenogens

Forest Clones

Seven of the 10 repeat types found in forest clones were also found in the cyclic parthenogens, including repeat types 1–4 (table 5). Repeat type 1 was fixed in five of the 12 forest clone isolates and was present in five others. Two repeat types found in the forest clones belonged to the "divergent" group of repeat types with the 750-bp insert. One (repeat type 29) was shared with the cyclic parthenogens, and the other (repeat type 28) was unique to a single forest clone. The average number of repeat types per forest clone isolate was 1.83 (table 2), which is significantly smaller than

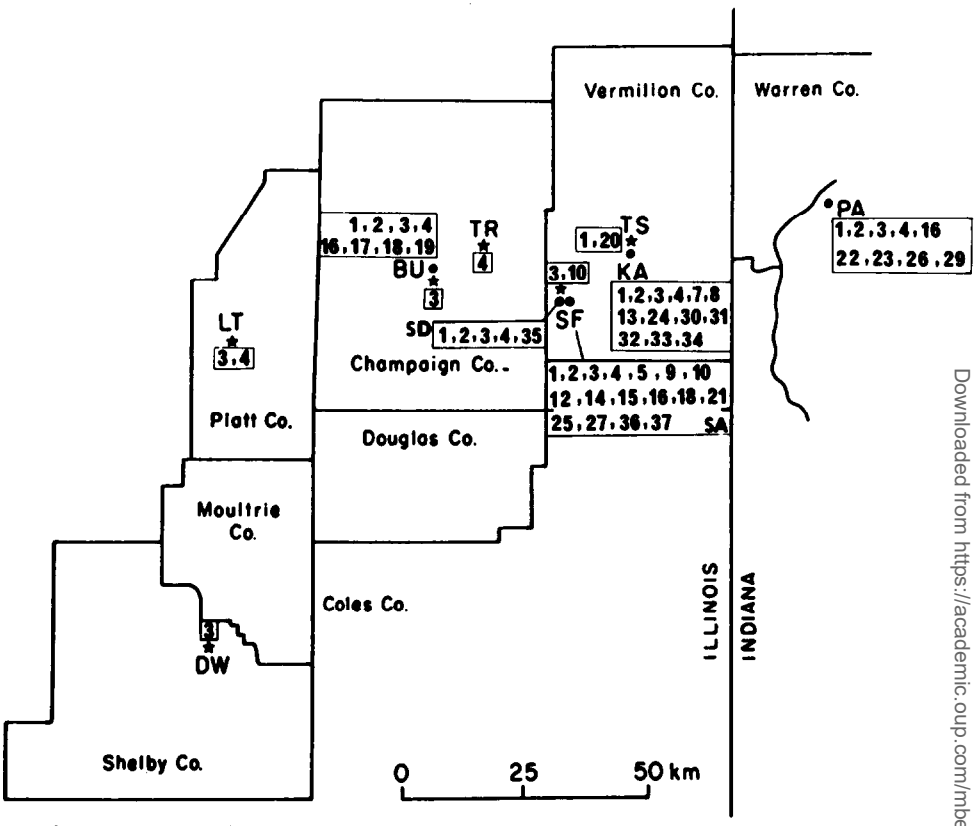

FIG. 4.—Map showing location of *Daphnia pulex* sampling sites in Illinois and Indiana, and complement of rDNA repeat types found at each site. The dots indicate cyclically parthenogenetic populations, and the stars indicate obligately parthenogenetic populations. BU contained both cyclic and obligate parthenogens. The star and box above SF correspond to the isolates taken from the three obligately parthenogenetic populations (SB, SP, and SW) on the SF. The two cyclic populations (SA and SD) are shown separately.

the average number of repeat types per cyclic isolate (2.61 ± 0.15; Student's $T_{(7)} = 5.29$, $P < 0.01$).

Urban Clones

Six of the urban clone isolates [1.5(TS), 2.4(LT), 2.6(BU), 4.1(DW), 4.1(SP), and 5.3(SP)] had repeats that were characteristic of both *D. pulex* and *D. pulicaria*, supporting the hypothesis that these clones are interspecific hybrids (Hebert et al. 1989). The *D. pulex* repeat types for these clones are shown in table 6 and figure 4. The *D. pulicaria* repeat types most commonly recognized in urban clones were $f1^{+}$-$h5^{-}$-$k5^{+}$-$r3^{+}$, $f1^{-}$-$h5^{-}$-$k5^{+}$-$r3^{+}$, and length variants of these. It is possible that recombinants between rDNA repeats of the two species were also present but could not be distinguished from nonrecombinants.

Three isolates [3.2(LT), 3.2(SB), and 3.2(SW)] appeared not to have any *D. pulicaria* repeats, as sites k5 and r3 were both absent. Though three *D. pulicaria* isolates were polymorphic for these two sites, no isolate lacked them completely. Conversely, two isolates [4.1(LT) and 4.1(TR)] appeared not to have any "pure" *D. pulex* repeats, as they were fixed for site r3, which is nearly fixed in *D. pulicaria* but

Table 2
rDNA Repeat-Type Variation in Cyclic Parthenogens and Forest Clones of *Daphnia pulex*

Population	No. of Individuals	Mean ± SE No. of Repeat Types/ Individual	No. of Repeat Types/ Population
KA	15	2.53 ± 0.36	13
BU	7	4.00 ± 0.38	8
PA	12	2.67 ± 0.36	9
SD	12	2.58 ± 0.23	5
SA	21	2.19 ± 0.26	17
Pooled cyclic parthenogens	67	2.61 ± 0.15	33
Forest clones	12	1.83 ± 0.32	10

is only found in one rare repeat type in *D. pulex*. These two clones were polymorphic for site k5, suggesting that the $k5^{-}$-$r3^{+}$ repeats are *D. pulex–D. pulicaria* recombinants.

In two cases, multiple isolates that had the same isozyme and mtDNA genotypes (3.2 and 4.1) and that were from different populations were analyzed. All three isolates with genotype 3.2 appeared to have the same rDNA profile. However, the four isolates with genotype 4.1 differed from one another (table 6 and fig. 4). If all of these isolates belong to the same clonal lineage (i.e., are not the result of independent hybridization events), then these changes can only be explained by the occurrence of recombination within and between rDNA arrays in the obligate parthenogens after the loss of meiosis.

To further test this hypothesis, 10 isolates of one genotype (1.5) that were from the same population (TS) were analyzed. Though all of the isolates were the same with respect to isozyme and mtDNA markers, the relative frequencies of the rDNA repeat types present varied among individuals (fig. 5).

Discussion

The results of the present study show that rDNA variation in *Daphnia pulex* is extensive. Of 73 mapped restriction sites, 31.5% were polymorphic. On the basis of restriction-site polymorphism alone, 23 rDNA repeat types were recognized in a sample of 90 isolates from Illinois, Indiana, and Ontario. To date, such a high degree of restriction-site polymorphism in rDNA has only been observed in one other organism, the locust (*Locusta migratoria*) (Schäfer and Kunz 1985).

It is clear that recombination within rDNA repeats has played an important role

Table 3
Estimates of rDNA Variation in Cyclically Parthenogenetic Populations of *Daphnia pulex*

Population	Mean ± SE rDNA Variation within Individuals	Mean ± SE rDNA Variation between Individuals	N_{IS} ± SE	Mean ± SE rDNA Variation within Populations
KA	0.0023 ± 0.0011	0.0012 ± 0.0015	0.35 ± 0.27	0.0034 ± 0.0012
PA	0.0032 ± 0.0014	0.0016 ± 0.0016	0.34 ± 0.20	0.0047 ± 0.0013
BU	0.0016 ± 0.0010	0.0005 ± 0.0008	0.24 ± 0.23	0.0021 ± 0.0010
SA	0.0010 ± 0.0006	0.0014 ± 0.0016	0.59 ± 0.30	0.0021 ± 0.0011
SD	0.0016 ± 0.0010	0.0004 ± 0.0009	0.21 ± 0.32	0.0019 ± 0.0008

NOTE.—All data are in substitutions/nucleotide site.

Table 4
Hierarchical Partitioning of rDNA Variation in Cyclically Parthenogenetic Populations of *Daphnia pulex*

Level of Analysis	Mean ± SE rDNA Variation	*N*-Statistic[a]
Population:		
Within individuals	0.0026 ± 0.0004	
Between individuals	0.0007 ± 0.0005	$N_{IS} = 0.34 \pm 0.11$
Locale (SF):		
Within populations:	0.0020 ± 0.0008	
Between populations	0.0003 ± 0.0007	$N_{SL} = 0.13 \pm 0.00$
Regional:		
Within locales	0.0030 ± 0.0010	$N_{SR} = 0.18 \pm 0.16$
Between locales	0.0007 ± 0.0008	$N_{LR} = 0.06 \pm 0.05$

[a] The value of N_{IS} is the mean for the five cyclic populations (table 3). The value of N_{SL} is based on the two populations located on SF. The regional values of within- and between-locale variation are the average of two calculations each involving PA, KA, BU, and one of the SF populations.

in the generation of much of this repeat-type diversity. For example, all possible combinations of the presence/absence of sites h5, k1, and h6 accounted for eight of the 23 repeat types.

Two types of length heterogeneity also contributed to rDNA variability in *D. pulex*. One type involved changes in the number of subrepeats in the IGS. In some species, rDNA spacer length variation is extensive. For example, a single individual of the broad bean (*Vicia faba*) can have as many as 20 size classes of rDNA repeats (Rogers et al. 1986). Conversely, little or no length heterogeneity has been detected in some species of yeast (Meyerink and Retèl 1976; Nath and Bollon 1977), sea urchin (Passananti et al. 1983), rye (Appels et al. 1980), and a variety of insects (Manning et al. 1978; Renkawitz et al. 1979; Schmidt et al. 1982; Schäfer and Kunz 1985). The level of IGS subrepeat array length variation in *D. pulex* was intermediate to these two extremes, as no individual had more than three size classes of rDNA repeats (at the level of resolution of the technique used), and in no case was the standard length repeat, 13.4 kb, missing. Indeed, the majority of repeats in most individuals were of this length. The predominance of one IGS size class within and among populations

Table 5
rDNA Repeat-Type Complements of *Daphnia pulex* Forest Clone Isolates

	rDNA Repeat Types Present in Clone Number[a]										
13 28	16 6	18 9	21 31	22 20	22 30	24 14	25 19	30 4	35 34	36 35	6 7
1 6	1 29	1	1	1 3 10 11	1 2	3 10	1	1	1 3 5 28	1	4

[a] The top number refers to the isolate's isozyme genotype, and the bottom number refers to its mtDNA genotype.
[b] Repeat-type numbers correspond to those listed in table 1.

Table 6
rDNA Repeat-Type Complements of *Daphnia pulex* Hybrid Urban Clone Isolates

rDNA Repeat Types Present in Clone Number										
1.5(TS)	3.2(LT)	3.2(SB)	3.2(SW)	2.4(LT)	2.6(BU)	4.1(LT)	4.1(TR)	4.1(DW)	4.1(SP)	5.3(SP)
1 20	3	3	3	4	3	*	*	3	3 10	3

NOTE.—Only rDNA repeat types from *D. pulex* are shown in the table. An asterisk indicates that the repeat types within this isolate were a mixture of *D. pulicaria* types and of *D. pulex–D. pulicaria* recombinant types. Repeat-type numbers correspond to those listed in table 1.

has also been observed in other organisms (Saghai-Maroof et al. 1984; Flavell et al. 1986; Williams et al. 1987). This pattern may reflect a relatively recent origin for the length variants detected in this survey, or, alternatively, there may be stabilizing selection in favor of repeats of a certain length. Williams et al. (1987) argued that selection was most likely responsible for the predominance of a particular size class on the X-chromosomal rDNA array of *Drosophila melanogaster*.

The other type of length heterogeneity found in *D. pulex* rDNA was the presence of a 750-bp insertion near the 3′ end of the 28S coding region. Inserts not related to subrepeat arrays have been found in a similar location in *Bombyx mori* (Lecanidou et al. 1984). Flavell et al. (1986) found length variation not associated with the IGS subrepeat array in *Triticum dicoccoides* but did not localize it further. One possibility is that the *D. pulex* insert is a transposon. Studies are currently underway to sequence this insert and to determine whether it is present at other locations in the genome.

When length heterogeneity and restriction-site polymorphism are considered si-

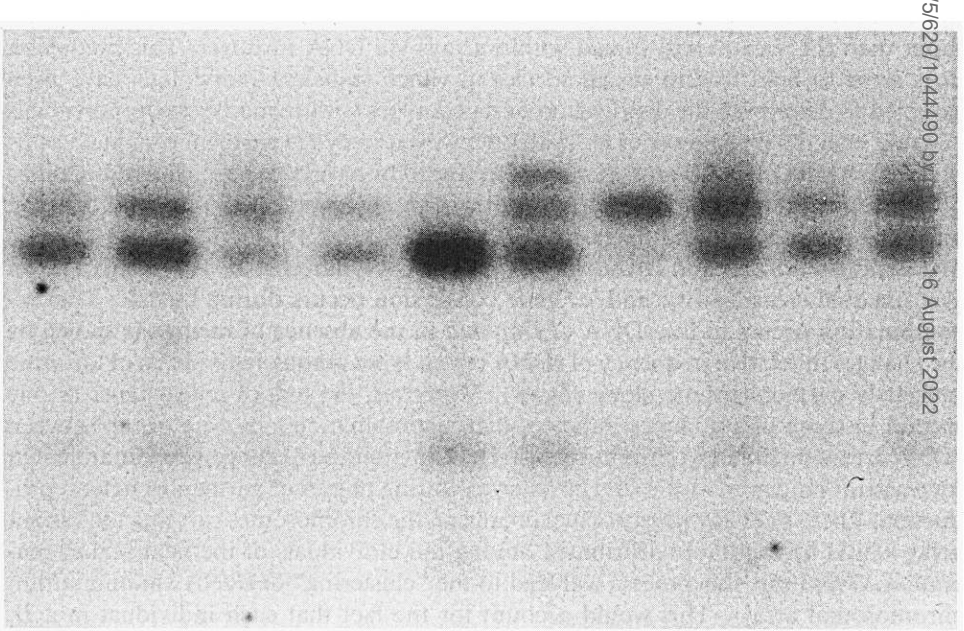

FIG. 5.—Southern blot analysis of repeat-type frequency within one obligately parthenogenetic hybrid urban clone. All individuals were taken from TS and have the isozyme and mtDNA genotype of urban clone 1.5. The DNA in each lane was digested with *Bst*EII. The Southern blot was probed with I-19.

multaneously, we find that the repeat types most divergent with respect to site variation also contain length variation. In the case of repeat types 27–29, three of the four restriction-site differences characterizing this group occurred very near the location of the 750-bp insert. Since this insert appears not to be homologous to any other region of the IGS, it may interfere with recombination between the IGS of repeats with and without it. Thus, repeats containing inserts may accumulate sequence variation that does not spread to other repeats. On the other hand, the length variation associated with the divergent repeat types 30–36 mapped to the region of IGS subrepeats. In each case, the inserts were >1 kb, a size which would include three to five additional subrepeats, depending on their size (200 bp or 330 bp). It is not clear why, if the length variants associated with them are due to changes in subrepeat array size, this group of repeat types has accumulated so many restriction site-differences. That recombination occurs between subrepeat arrays of repeat types within the group is suggested by the fact that several of the repeat types (i.e., 30–34) differ only with respect to their length. Perhaps recombination is also restricted between subrepeat arrays that differ greatly in size, causing some very long repeats to evolve independently. However, nothing of this nature has been reported for other organisms. Another possibility is that the inserts in this repeat-type family contain sequences that are not IGS subrepeats. This would create a situation similar to that in repeat types 27–29.

Although a large number of repeat types were recognized in the present study, many of them had very limited distributions, and over half were found in only a single individual. However, these rare repeat types were sometimes present in high frequency within individuals, suggesting that new variants spread through rDNA arrays faster than the arrays carrying the variant spread through populations. This is contrary to a model developed by Ohta and Dover (1984) for obligately sexual organisms. Their model predicts that there will not be large differences between individuals in the copy number of a variant gene as it spreads through a sexual population, because the chromosomes carrying the variant will spread among individuals via sexual reproduction faster than the variant will spread within arrays via DNA turnover. This prediction does seem to hold true in sexual species in which sufficient individuals have been sampled to determine the distribution of repeat types within and between individuals (Suzuki et al. 1987; Seperak et al. 1988). However, in cyclic parthenogens such as *D. pulex*, each bout of sexual reproduction is separated by a variable number of generations of ameiotic parthenogenesis. Genetic studies of yeast (Szostak and Wu 1980; Jackson and Fink 1981; Klein and Petes 1981; Keil and Roeder 1984), *Vicia faba* (Rogers and Bendich 1987*a*), and *Drosophila* (Tartof 1984; Gillings et al. 1987) have shown that unequal crossing-over and/or gene conversion occurs during mitosis. That recombination occurs in the rDNA of *Daphnia* in the absence of meiosis is shown by the changes in relative frequency of rDNA repeat types among individuals of the same obligately parthenogenetic clone (fig. 5). Moreover, the loss of repeat types of one species in some urban clones indicates that recombination can even occur between rDNA arrays on different chromosomes. Thus, in cyclic parthenogens, a variant could increase in frequency within an rDNA array during phases of parthenogenetic reproduction. Then, as in any purely sexual organism, the chromosomes carrying the variant array would be randomly distributed among the individuals of the next sexual generation. Over time, this process will lead to the "clustering" of rDNA variants within chromosomal arrays. This would account for the fact that each individual in a *D. pulex* population carries only a small proportion of the variation present in the population as a whole (table 3), as would be the case for a single-copy locus with many alleles.

Clustering of rDNA variants within chromosomes has been observed in other organisms, particularly with respect to IGS length variants (Arnheim et al. 1982; Rogers and Bendich 1987*a*). Seperak et al. (1988) argued that such clustering will occur even in sexual organisms if intrachromosomal DNA exchanges are much more frequent than interchromosomal exchanges.

Estimates of rDNA variation (average number of substitutions per nucleotide site between random pairs of haplotypes) within cyclically parthenogenetic populations of *D. pulex* were similar to those calculated for single-copy genes in *Drosophila* (Lynch and Crease 1990). These estimates range from 0.0003 for the *yellow-achaete-scute* region in *D. melanogaster* (Aguadé et al. 1989) to 0.0064 for *alcohol dehydrogenase* in *D. melanogaster* (Aquadro et al. 1986). The mean within-population variation for rDNA in *D. pulex* was 0.0028, which is very similar to 0.0024, the mean within-population variation for mtDNA in this species (Crease et al. 1990).

Despite the considerable rDNA variation within populations, there was less between-population variation at either the local or regional levels (table 4). However, these estimates are downwardly biased, for reasons discussed above. Crease et al.'s survey of mtDNA and isozyme variation in *D. pulex* revealed considerable between-populations differentiation at the regional level, for both types of loci. On the basis of the results of the present study, rDNA appears to be diverging almost as rapidly as single-copy isozyme loci. It was suggested that the limited dispersal ability of *D. pulex* is an important factor contributing to this differentiation. The disjunct distribution of the majority of rDNA repeat types sampled in the present study (table 1 and fig. 4) is consistent with the suggestion that gene flow among demes is limited.

The few comparisons of isozyme and rDNA variation within-species that have been made have yielded results that are somewhat contrary to those found in *D. pulex*. For example, Suzuki et al. (1987) found that, among the karyotypic races of the mole rat (*Spalax enhrenbergi*), differences at isozyme loci arose primarily from changes in relative frequencies of alleles but that each race was characterized by a different complement of rDNA repeat types. This led Suzuki et al. to suggest that rDNA evolves at a much faster rate than other nuclear loci. Sites and Davis (1989) found a similar result in their study of rDNA and isozyme variation in chromosomal races of the lizard *Sceloporus grammicus* from Mexico.

In contrast to their cyclic relatives, obligately asexual forest clone isolates of *D. pulex* carried fewer rDNA repeat types, on average. Although novel repeat types will undoubtedly arise via mutation in these clones, recombination within and between rDNA arrays in the absence of sexual reproduction appears to lead, overall, to a net loss of rDNA variation within individuals. The fact that many of the forest isolates were fixed (or nearly so) for repeat types that are common in the cyclic parthenogens most likely reflects their recent origin from a cyclic ancestor (Crease et al. 1989, 1990).

The urban clones arose from hybridization between two species and thus started out with a high frequency of at least two different rDNA repeat types. In the absence of selection, the relative frequencies of rDNA repeat types within individuals of obligate clones are expected to diverge from one another as each lineage is fixed for a different repeat type. Such a pattern was in fact observed among genotype 4.1 isolates from different populations and among genotype 1.5 isolates from TS.

The homogenization of rDNA arrays during ameiotic reproduction has important evolutionary implications for the obligate parthenogens. For example, it may provide a mechanism by which they can retard or even escape Muller's (1964) ratchet, by allowing them to purge the gene family of deleterious variants. Muller's ratchet refers to the idea that recessive deleterious mutations will accumulate in the genomes of

parthenogenetic organisms. Conversely, the fixation of favorable new variants may also provide opportunities for evolution within established clones. For example, changes in rDNA are known to have substantial effects on the morphology and life history of *Drosophila* (Frankham 1988). Thus, it is of interest that isolates of isozyme/mtDNA genotype 4.1 from different populations which differ significantly with respect to life-history characters (Lynch et al. 1989) can also be distinguished on the basis of their rDNA.

Although members of multigene families within species are generally more similar to one another than to members of the same family in other species, ancestral polymorphisms can persist within closely related species. Williams et al. (1988) argued that such polymorphisms may cause problems in the construction of phylogenies when only one or a few individuals from each species are analyzed. In the present study, three restriction-site polymorphisms were shared between *D. pulex* and *D. pulicaria*. Site f1 was usually polymorphic within as well as between individuals in both species. On the other hand, site k1 was present in all repeats of some *D. pulex* individuals and completely lacking in other individuals. A similar situation was also observed for site h5 in both species. Thus, the presence/absence of these sites could erroneously be observed as fixed differences between the species, depending on the individuals chosen for study.

Daphnia pulex and *D. pulicaria* appear to be very closely related to one another on the basis of rDNA (present study) and isozymes (Hebert et al. 1989; P. D. N. Hebert, personal communication). Thus, the discovery of shared rDNA site polymorphisms is perhaps not too surprising. However, one polymorphism (presence/absence of site i5) was shared between *D. pulicaria* and *D. obtusa* (fig. 1), two species which have diverged substantially (Benzie 1986). Such polymorphisms are not expected in more distantly related species unless they arose by convergence (Hillis and Davis 1988) or by hybridization.

The small amount of shared polymorphism detected among these three species would be unlikely to cause problems in a phylogenetic analysis. The close relationship between *D. pulex* and *D. pulicaria* compared with *D. obtusa* would not be obscured by the homoplasy due to polymorphic sites. However, it is worth noting that such polymorphisms do, in fact, exist, and care should be taken to determine levels of intraspecific variation before using interspecific variation in multigene families to infer phylogenetic relationships.

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LITERATURE CITED

AQUADÉ, M., N. MIYASHITA, and C. LANGLEY. 1989. Reduced variation in the *yellow-achaete-scute* region in natural populations of *Drosophila melanogaster*. Genetics **122**:607–615.

APPELS, R., W. L. GERLACH, E. S. DENNIS, H. SWIFT, and W. J. PEACOCK. 1980. Molecular

and chromosomal organization of DNA sequences coding for the ribosomal RNAs in cereals. Chromosoma **78**:293–311.

AQUADRO, C. F., S. F. DESSE, M. M. BLAND, C. H. LANGLEY, and C. C. LAURIE-AHLBERG. 1986. Molecular population genetics of the alcohol dehydrogenase gene region of *Drosophila melanogaster.* Genetics **114**:1165–1190.

ARNHEIM, N. 1983. Concerted evolution of multigene families. Pp. 38–61 *in* M. NEI and R. K. KOEHN, eds. Evolution of genes and proteins. Sinauer, Sunderland, Mass.

ARNHEIM, N., D. TRECO, B. TAYLOR, and E. M. EICHER. 1982. Distribution of ribosomal gene length variants among mouse chromosomes. Proc. Natl. Acad. Sci. USA **79**:4677–4680.

BENZIE, J. A. H. 1986. Phenetic and cladistic analyses of the phylogenetic relationships within the genus *Daphnia* worldwide. Hydrobiologia **140**:105–124.

BOUSQUET, J., E. GIROUARD, C. STROBECK, B. P. DANCIK, and M. LALONDE. 1989. Restriction fragment length polymorphisms in the rDNA region among seven species of *Alnus* and *Betula papyrifera.* Plant Soil **118**:231–240.

CLARK, A. G. 1990. Inference of haplotypes from PCR-amplified samples of diploid populations. Mol. Biol. Evol. **7**:111–122.

COEN, E. S., T. STRACHAN, and G. A. DOVER. 1982. Dynamics of concerted evolution of ribosomal DNA and histone gene families in the *melanogaster* species subgroup of *Drosophila.* J. Mol. Biol. **158**:17–35.

CREASE, T. J. 1986. Mitochondrial DNA variation in *Daphnia pulex* Leydig reproducing by obligate and cyclic parthenogenesis. Ph.D. diss., Washington University, St. Louis.

CREASE, T. J., M. LYNCH, and K. SPITZE. 1990. Hierarchical analysis of population genetic variation in mitochondrial and nuclear genes of *Daphnia pulex.* Mol. Biol. Evol. **7**:444–458.

CREASE, T. J., D. J. STANTON, and P. D. N. HEBERT. 1989. Polyphyletic origin of asexuality in *Daphnia pulex.* II. Mitochondrial DNA variation. Evolution **43**:1016–1026.

DOVER, G. A. 1982. Molecular drive: a cohesive mode of species evolution. Nature **299**:111–117.

FEINBERG, A. P., and B. VOGELSTEIN. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.

FIELD, K. G., G. J. OLSEN, D. J. LANE, S. J. GIOVANNONI, S. J. GHISELIN, E. C. RAFF, N. R. PACE, and R. A. RAFF. 1988. Molecular phylogeny of the animal kingdom. Science **239**:748–752.

FLAVELL, R. B., M. O'DELL, P. SHARP, E. NEVO, and A. BEILES. 1986. Variation in the intergenic spacer of ribosomal DNA of wild wheat, *Triticum dicoccoides,* in Israel. Mol. Biol. Evol. **3**:547–558.

FRANKHAM, R. 1988. Exchanges in the rDNA multigene family as a source of genetic variation. Pp. 236–242 *in* B. S. WEIR, E. J. EISEN, M. M. GOODMAN, and G. NAMKOONG, eds. Proceedings of the Second International Conference on Quantitative Genetics. Sinauer, Sunderland, Mass.

GERBI, S. A. 1985. Evolution of ribosomal DNA. Pp. 419–517 *in* R. J. MACINTYRE, ed. Molecular evolutionary genetics. Plenum, New York.

GILLINGS, M. R., R. FRANKHAM, J. SPEIRS, and M. WHALLEY. 1987. X-Y exchange and the coevolution of the X and Y rDNA arrays in *Drosophila melanogaster.* Genetics **116**:241–251.

HARTL, D. 1980. Principles of population genetics. Sinauer, Sunderland, Mass.

HASEGAWA, M., Y. IIDA, T. YANO, F. TAKAIWA, and M. IWABUCHI. 1985. Phylogenetic relationship among eukaryotic kingdoms inferred by ribosomal RNA sequences. J. Mol. Evol. **22**:32–38.

HEBERT, P. D. N. 1981. Obligate asexuality in *Daphnia.* Am. Nat. **117**:784–789.

HEBERT, P. D. N., M. J. BEATON, and S. S. SCHWARTZ. 1989. Polyphyletic origin of asexuality in *Daphnia pulex.* I. Breeding system variation and levels of clonal diversity. Evolution **43**:1004–1015.

HEBERT, P. D. N., and T. J. CREASE. 1980. Clonal coexistence in *Daphnia pulex* (Leydig): another planktonic pardox. Science **207**:513–525.

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

HILLIS, D. M., and S. K. DAVIS. 1986. Evolution of ribosomal DNA: fifty million years of recorded history in the frog genus *Rana*. Evolution **40**:1275–1288.

———. 1988. Ribosomal DNA: intraspecific polymorphism, concerted evolution, and phylogeny reconstruction. Syst. Zool. **37**:63–66.

HINTZ, W. E. A., J. B. ANDERSON, and P. A. HORGEN. 1989. Relatedness of three species of *Agaricus* inferred from restriction fragment length polymorphism analysis of the ribosomal DNA repeat and mitochondrial DNA. Genome **32**:173–178.

JACKSON, J. A., and G. FINK. 1981. Gene conversion between duplicated elements in yeast. Nature **292**:306–307.

KEIL, R. L., and G. S. ROEDER. 1984. *Cis*-acting recombination-stimulating activity in a fragment of the ribosomal DNA of *Saccharomyces cerevisiae*. Cell **39**:377–386.

KING, L. M., and B. A. SCHAAL. 1989. Ribosomal-DNA variation and distribution in *Rubeckia missouriensis*. Evolution **43**:1117–1119.

KLEIN, H. L., and T. PETES. 1981. Intrachromosomal gene conversion in yeast: a new type of genetic exchange. Nature **289**:144–148.

LEARN, G. H., JR., and B. A. SCHAAL. 1987. Population subdivision for ribosomal DNA repeat variants in *Clematis fremontii*. Evolution **41**:433–438.

LECANIDOU, R., T. H. EICKBUSH, and F. C. KAFATOS. 1984. Ribosomal DNA genes of *Bombyx mori:* a minor fraction of the repeating units contain insertions. Nucleic Acids Res. **12**:4703–4713.

LONG, E. O., and I. B. DAWID. 1980. Repeated genes in eukarytoes. Annu. Rev. Biochem. **49**:727–764.

LYNCH, M. 1987. The consequences of fluctuating selection for isozyme polymorphisms in *Daphnia*. Genetics **115**:657–669.

LYNCH, M., and T. J. CREASE. 1990. The analysis of population survey data on DNA sequence variation. Mol. Biol. Evol. **7**:377–394.

LYNCH, M., K. SPITZE, and T. J. CREASE. 1989. The distribution of life-history variation in the *Daphnia pulex* complex. Evolution **43**:1724–1736.

MCLAIN, D. K., and F. H. COLLINS. 1989. Structure of rDNA in the mosquito *Anopheles gambiae* and rDNA sequence variation within and between species of the *A. gambiae* complex. Heredity **62**:233–242.

MCLAIN, D. K., F. H. COLLINS, A. D. BRANDLING-BENNETT, and J. B. O. WERE. 1989. Microgeographic variation in rDNA intergenic spacers of *Anopheles gambiae* in western Kenya. Heredity **62**:257–264.

MANIATIS, T., E. FRITSCH, and J. SAMBROOK. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MANNING, R. F., D. R. SAMOLS, and L. P. GAGE. 1978. The genes for 18S, 5.8S and 28S ribosomal RNA of *Bombyx mori* are organized into tandem repeats of uniform length. Gene **4**:153–166.

MEYERINK, J. H., and J. RETÈL. 1976. Topographical analysis of yeast ribosomal DNA by cleavage with restriction endonucleases. II. The physical map of *Eco* RI fragments. Nucleic Acids Res. **3**:2697–2707.

MINDELL, D. P., and R. L. HONEYCUTT. 1989. Variability in transcribed regions of ribosomal DNA and early divergences in birds. Auk **106**:539–548.

MOLNAR, S. J., and G. FEDAK. 1989. Polymorphism in ribosomal DNA repeat units of 12 *Hordeum* species. Genome **32**:1124–1127.

MULLER, H. J. 1964. The relation of recombination to mutational advance. Mutat. Res. **1**:2–9.

NATH, K., and A. P. BOLLON. 1977. Organization of the yeast ribosomal RNA gene cluster via cloning and restriction analysis. J. Biol. Chem. **252**:6562–6571.

NEI, M. 1986. Definition and estimation of fixation indices. Evolution **40**:643–645.

NEI, M., and F. TAJIMA. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics **105**:207–217.

NELLES, L., B.-L. FANG, G. VOLKAERT, A. VANDENBERGHE, and R. DEWACHTER. 1984. Nucleotide sequence of a crustacean 18S ribosomal RNA gene and secondary structure of eukaryotic small subunit ribosomal RNAs. Nucleic Acids Res. **12**:8749–8768.

OHTA, T., and G. A. DOVER. 1984. The cohesive population genetics of molecular drive. Genetics **108**:501–521.

PASSANANTI, C., A. FELSANI, R. GIORDANO, S. METAFORA, and C. SPADAFORA. 1983. Cloning and characterization of the ribosomal genes of the sea-urchin *Paracentrotus lividus.* Eur. J. Biochem. **13**:233–239.

RENKAWITZ, R., S. A. GERBI, and K. H. GLATZER. 1979. Ribosomal DNA of the fly *Sciara coprophila* has a very small and homogeneous repeat unit. Mol. Gen. Genet. **173**:1–13.

ROBERTS, M. P., and J. C. VAUGHN. 1982. Ribosomal RNA sequence conservation and gene number in the larval brine shrimp. Biochim. Biophys. Acta **697**:148–155.

ROGERS, S. O., and A. J. BENDICH. 1987*a*. Heritability and variability in ribosomal RNA genes of *Vicia faba.* Genetics **117**:285–295.

———. 1987*b*. Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Mol. Biol. **9**:509–520.

ROGERS, S. O., S. HONDA, and A. J. BENDICH. 1986. Variation in the ribosomal RNA genes among individuals of *Vicia faba.* Plant Mol. Biol. **6**:339–345.

SAGHAI-MAROOF, M. A., K. M. SOLIMAN, R. A. JORGENSEN, and R. W. ALLARD. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA **81**:8014–8018.

SCHAEFFER, S. W., C. F. AQUADRO, and W. W. ANDERSON. 1987. Restriction-map variation in the alcohol dehydrogenase region of *Drosophila pseudoobscura.* Mol. Biol. Evol. **4**:254–265.

SCHÄFER, M., and W. KUNZ. 1985. rDNA in *Locusta migratoria* is very variable: two introns and extensive restriction site polymorphisms in the spacer. Nucleic Acids Res. **13**:1251–1266.

SCHAFFER, H. E., and R. R. SEDEROFF. 1981. Improved estimation of DNA fragment lengths from agarose gels. Anal. Biochem. **115**:113–122.

SCHMIDT, E. R., E. A. GODWIN, H.-G. KEYL, and N. ISRAELEWSKI. 1982. Cloning and analysis of ribosomal DNA of *Chironomus thummipiger* and *Chironomus thummi thummi:* the nontranscribed spacer of *Chironomus thummi thummi* contains a highly repetitive DNA sequence. Chromosoma **87**:389–407.

SEPERAK, P., M. SLATKIN, and N. ARNHEIM. 1988. Linkage disequilibrium in human ribosomal genes: implications for multigene family evolution. Genetics **119**:943–949.

SITES, J. W., JR., and S. K. DAVIS. 1989. Phylogenetic relationships and molecular variability within and among six chromosome races of *Sceloporus grammicus* (Sauria, Iguanidae), based on nuclear and mitochondrial markers. Evolution **43**:296–317.

SUZUKI, H., N. MIYASHITA, K. MORIWAKI, R. KOMINAMI, M. MURAMATSU, T. KANEHISA, F. BONHOMME, M. L. PETRAS, Z.-C. YU, and D.-Y. LU. 1986. Evolutionary implication of heterogeneity of the nontranscribed spacer region of ribosomal DNA repeating units in various subspecies of *Mus musculus.* Mol. Biol. Evol. **3**:126–137.

SUZUKI, H., K. MORIWAKI, and E. NEVO. 1987. Ribosomal DNA (rDNA) spacer polymorphism in mole rats. Mol. Biol. Evol. **4**:602–610.

SYTSMA, K. J., and B. A. SCHAAL. 1985. Phylogenetics of the *Lisianthus skinneri* (Gentianaceae) species complex in Panama utilizing DNA restriction fragment analysis. Evolution **39**:594–608.

SZOSTAK, J. W., and R. WU. 1980. Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae.* Nature **284**:426–430.

TARTOF, K. D. 1984. Unequal mitotic sister chromatid exchange and disproportionate replication as mechanisms regulating ribosomal RNA gene redundancy. Cold Spring Harbor Symp. Quant. Biol. **38**:491–500.

VERMA, M., and S. K. DUTTA. 1987. Phylogenetic implication of heterogeneity of the non-

transcribed spacer of rDNA repeating unit in various *Neurospora* and related fungal species. Curr. Genet. **11**:309–314.

VOSSBRINCK, C. R., and S. FRIEDMAN. 1989. A 28S ribosomal RNA phylogeny of certain cyclorrhaphous Diptera based upon a hypervariable region. Syst. Entomol. **14**:417–431.

WALSH, J. B. 1987. Persistence of tandem arrays: implications for satellite and simple-sequence DNAs. Genetics **115**:553–567.

WILLIAMS, S. M., R. W. DEBRY, and J. L. FEDER. 1988. A commentary on the use of ribosomal DNA in systematic studies. Syst. Zool. **37**:60–62.

WILLIAMS, S. M., G. R. FURNIER, E. FUOG, and C. STROBECK. 1987. Evolution of the ribosomal DNA spacers of *Drosophila melanogaster:* different patterns of variations on X and Y chromosomes. Genetics **116**:225–232.

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