

Factors organising and maintaining polymorphism in a cyclic parthenogenetic species: *Daphnia pulex*

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The possible causes of the evolutionary stability of cyclic parthenogenesis are considered. The large genotypic variability of *Daphnia pulex* clones for reproduction systems in natural populations is provided by the high probability of interclonal crosses. When reproduction is mainly parthenogenetic, the less adapted clones are eliminated, and balanced interclonal polymorphism is established among the survivors. The following selective forces maintain polymorphism for mode of reproduction: high reproduction efficiency of clones prone to parthenogenesis, on the one hand, short-term advantage (high resistance to crowding and unfavourable conditions) of those prone to bisexuality, on the other hand.

This selection gives rise to a stable polymorphism maintained by several developmental programmes within a genotype. Activation of any of the programmes and of the corresponding gene groups depends on genotypic and environmental backgrounds.

INTRODUCTION

During the past decade *Daphnia pulex* has acquired the status of an advantageous research tool. Thus interest in typical inhabitants of fresh water reservoirs has accrued (Hebert *et al.* 1972; Hebert, 1978; Young, 1979). *Daphnia pulex*, like many other Cladoceran species, has a unique reproduction system adapted in relation to its habitat. This system is based on alternating recourse to ameiotic parthenogenesis and bisexuality (Banta, 1939). The long co-existence of both modes of reproduction during evolution raised questions concerning their underlying mechanisms (Maynard Smith, 1978). One of the major consequences of parthenogenetic reproduction in *Daphnia* is the organisation of a special population structure with a relatively small number of clones (Hebert and Crease 1980). This paper is concerned with factors maintaining clonal diversity in *Daphnia pulex* populations and the related polymorphism in reproduction systems.

MATERIAL AND METHODS

Daphnia pulex from shallow ponds of the Novosibirsk region were used in the experiments.

* Deceased 14 November 1985.

The laboratory clones derived from these populations were reared under standard conditions in bottles. To determine differences in fitness components, 1-2 day-old females from the same brood were placed in 250 ml bottles singly (low density population) or in groups of eight (high density population). All the animals remained in the bottles to adulthood. To evaluate the reproductive capacity of adult females, the number and sex of newborns were determined, and sexual eggs, when appearing, were removed. The experiments were done in 8-10 replicates for the low density cultures and in 5-8 replicates for the high density ones. Fecundity F was expressed as product of the number of young produced divided by the product of the number of adult females per bottle and the time elapsed after each count, i.e. $F = V/nt$, where F denotes fecundity, V the number of young counted, n the number of adult females per bottle, t the number of days after each consecutive count.

Artificial populations were set up in 5 litre bottles containing culture medium into which 30 newborn females from each clone were added. The number of *Daphnia* in a sample containing 10 per cent of the volume of the culture medium was counted, and thus the total number of animals per bottle was determined. To calculate the proportion of each clone in a culture, 75-100 individuals were

taken at random every 7–10 days for electrophoresis. The sex of each individual was recorded before each electrophoretic procedure. This allowed us to calculate the percentage of males in each clone and the proportion of females from each clone in the artificial population.

Temporal changes in allele frequencies in the populations were studied by means of the method of polyacrylamide gel electrophoresis described elsewhere (Ruvinsky and Lobkov, 1981). The total number of electrophoretically examined individuals was more than 2300, and that counted in all the experiments was about 120,000.

EXPERIMENTS

Variations in clonal capacity for bisexual reproduction

Banta (1939) gave the first indication that clones differ in their capacity for bisexuality. Our aim was to identify clones contrasting in this capacity for subsequent studies. We detected clones marked with different esterase patterns. These clones also differed in reproductive characteristics. Table I presents the values for these characteristics averaged for 5–10 replicates.

We used a coefficient to calculate the suppression of parthenogenetic reproduction. This coefficient expressed the degree to which fecundity was decreased at high- compared with low-population densities, i.e. it was the ratio of fecundity at low density to fecundity at high density.

Decrease in the productivity of parthenogenetically reproducing females in response to increase in culture density was presumably either the result of lower fecundity or transition to sexual egg production. The data represented in Table I indicate that the clones differed markedly in all the five reproductive characteristics. Clonal differences were the largest between ZS-4 and Z-4, Ch-26. The majority of the clones varied in different charac-

teristics: capacity for production of sexual eggs and males, number of parthenogenetically produced offspring. However, the differences were less pronounced than those for the six established clones, and they concerned just one of the characteristics. In some clones, there was no correlation between their capacity for male production and laying of sexual eggs. We identified some other clones capable of producing males, but not laying sexual eggs and vice versa. Furthermore, other clones seemed to be incapable of passing to bisexual reproduction (ZS-4). There were yet other clones succeeding in this passage (Ch-26). We inferred that clones can contribute differently to bisexual and parthenogenetic reproduction.

Clonal competition in artificial populations

Artificial populations of *Daphnia* were established taking into account our previous results of analysis of monoclonal cultures (Ruvinsky *et al.*, 1985). Let us consider here a simplest artificial population consisting of two clones. The consequences of competition between a pair of clones are either competitive exclusion or stable co-existence of the competitors.

Fig. 1 shows how the artificial population changed with time. It was composed of clones K-24 and Ch-26 considerably differing in reproductive characteristics. No males were ever recovered in K-24, while they appeared regularly in Ch-26, even under optimum conditions. The rapid exclusion of clone Ch-26 from the artificial population presumably followed as a consequence of suppressed parthenogenesis and the appearance of males in large numbers. All the 112 females examined 48 days after the experiment was started exhibited the esterase patterns of clone K-24. In preliminary experiments, clone Z-4 with strongly suppressed parthenogenetic reproduction and propensity to bisexuality, when crowded (like clone Ch-26), was also excluded from the artificial populations. Thus,

Table 1 A characterisation of reproduction patterns in *Daphnia pulex* clones at high density.

Index	ZS-4	K-24	ZS-5	ZS-9	Z-4	Ch-26
Males %	0	0	0	4.1	17.7	51.7
Ephippial females %	0	0	0	3.5	13.8	23.0
Non-reproducing females %	3.0	—	6.7	6.8	22.9	30.0
Parthenogenetically reproducing females %	97.0	100	93.3	89.7	63.3	47.0
Suppression coefficient of parthenogenesis at high density*	1.41	2.32	1.89	2.04	3.57	3.85

* The coefficient is expressed as the ratio of fecundity at low density to that at high density.

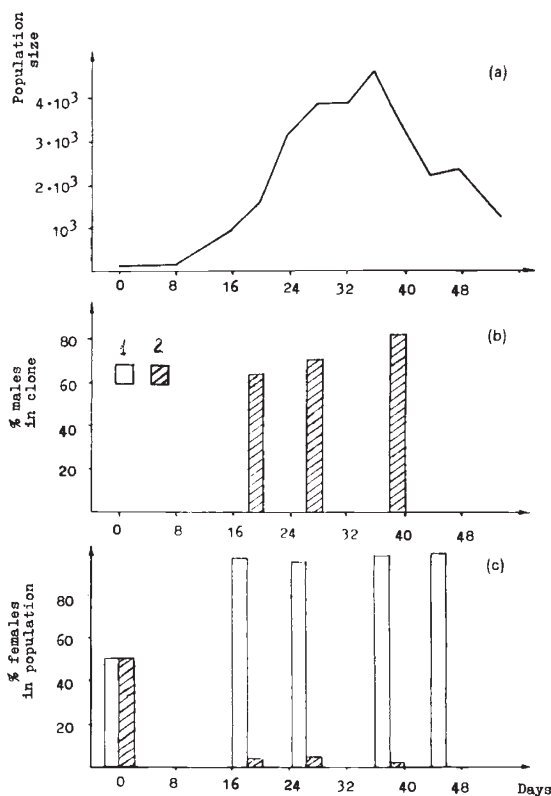


Figure 1 Time course of changes in population size (a) and number of males (b) and females (c) in the artificial population of *Daphnia pulex* composed of clones K-24 (1) and Ch-26 (2).

clones with high suppression coefficients of parthenogenetic reproduction can be rapidly eliminated from artificial populations (table I).

The second artificial population was composed of more typical clones ZS-5 and ZS-9, differing mainly in propensity for bisexuality at high densities (table I). Fig. 2(a) shows how the size of the population changed during the 72 days of observation; some of the values for parthenogenetic and bisexual reproduction are also entered in this figure. At the time when population size attained the first maximum, the proportion of females from ZS-5 was more than 80 per cent of the total number of females in the bottle (fig. 2(c)), while that of males from clone ZS-5 was 1.7 per cent of its number in the culture (fig. 2(b)). This is in contrast to the situation observed for clone ZS-9.

After the occurrence of the first maximum of population size, parthenogenetic reproduction was suspended to about day 56 of the experiment. High mortality was presumably the main cause of decrease in population size. Decline in total population size occurred concomitantly with sig-

nificant increase in the proportion of ZS-9 females ($P > 0.95$; $\chi^2 = 5.43$). In the absence of parthenogenesis, increase in the proportion of ZS-9 may be explained by its higher resistance to crowding effects compared to ZS-5. Decrease in population size of ZS-5-ZS-9 was followed by return to parthenogenetic reproduction on day 60. In a sample of 50 females, which started to reproduce parthenogenetically, 11 were from ZS-5 (22 per cent) and 39 from ZS-9 (78 per cent). However, the temporal changes in clonal structure (fig. 2(c)) shows that the proportion of females from ZS-5 was 65 per cent and that from ZS-9 was 35 per cent to day 60 of observation.

Therefore ZS-9 is more resistant to increase in population density, it has more readily recourse to parthenogenesis after population size decreases and, concomitantly, produces a significantly larger number of males, i.e., its tendency to bisexuality is stronger. Resumption of parthenogenetic reproduction culminated repeatedly in population size increase, but the second peak was 1.5 times smaller than the first. Increase in population size was, to a large extent, due to the reproduction of individuals from ZS-9 and, as a consequence, the difference between the frequencies of *Daphnia* from the competing clones decreased. Thus, clonal rivalry has one of the consequences: exclusion of some of the clones or establishment of a fluctuating equilibrium of the frequencies of the competing clones ensuring their prolonged co-existence.

Genetic structure of Daphnia pulex natural populations

In *Daphnia pulex*, esterases are coded for by three closely linked genes, the total number of, so far, identified alleles being 12 (Ruvinsky and Lobkov, 1981). Nine hundred different esterase genotypes are theoretically possible. This diversity is sufficient for analysis of the genetic structure of a *Daphnia* population. However, studies of natural populations revealed that they actually contain a small proportion of the possible genotypes. The esterase genotypes encountered in a typically intermittent population Z are shown in fig. 3: the Z vernal population possesses 14 esterase genotypes, of which three occur in more than 65 per cent of the total number of *Daphnia* examined; in autumn, by the end of the cycle of parthenogenetic reproduction, genotypic and, hence, clonal diversity decrease (fig. 3). Obviously, the clones prevailing in the population successfully coexist throughout the entire period of parthenogenetic reproduction. Thus reasoning, we examined

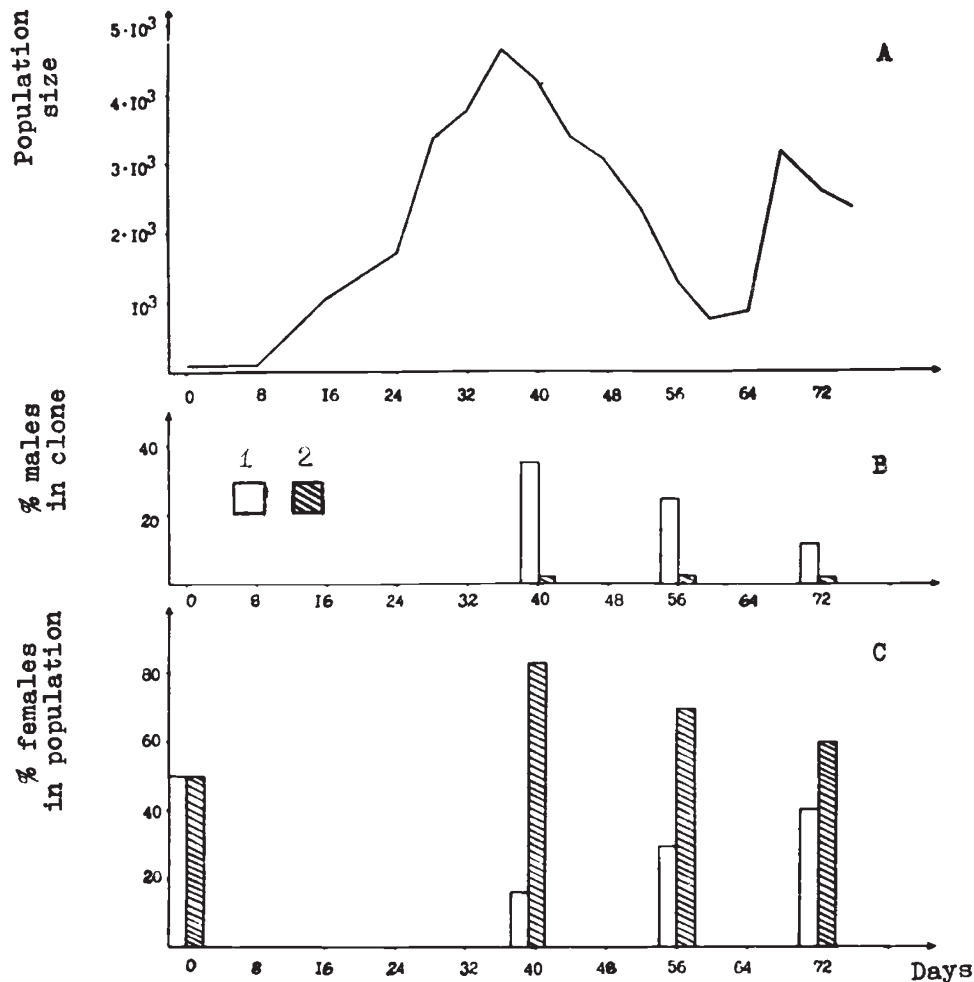


Figure 2 Time course of changes in population size (a) and number of males (b) and females (c) in the artificial population of *Daphnia pulex* composed of clones ZS-9 (1) and ZS-5 (2).

population Ch. described earlier (Ruvinsky *et al.*, 1985a). The population is an extreme case of intermittence conforming to extremely harsh environments; its parthenogenetic period is reduced to 4–6 weeks. Although the number of esterase genotypes detected in population Ch. exceeded severalfold that in a non-extreme, typically intermittent population, attaining 95, more than 50 per cent of *Daphnia* were marked with only 12 of the frequently occurring genotypes (fig. 4). Changes in the genotypic 1982 during the 45 days of their life is depicted in fig. 5. Clearly, some clones ceased to exist, others persisted. Although Ch. differs from the previously studied intermittent Z population in genotypic structure, it conforms to the same ecologo-genetical pattern: exclusion of some clones and co-existence of the rest in the population.

The narrowing of genotypic diversity in popu-

lation Z consequent to prolonged parthenogenesis is partly counteracted by clonal heterogeneity for propensity to bisexuality. The data of table 2 demonstrate that males and females of different genotypes contribute differently to bisexual reproduction. The genotypic frequencies in individuals of opposite sexes are very different. This inevitably makes more probable the occurrence of interclonal matings.

Thus analysis of the genetic structure of natural *Daphnia* populations revealed the following features: the ousting of certain genotypes (and of the corresponding clones), long co-existence of some other clones during parthenogenetic reproduction; differential involvement of genotypes in bisexual reproduction, stable reproduction of widespread genotypes in the next year and, ultimately, the establishment of new adapted genotypes.

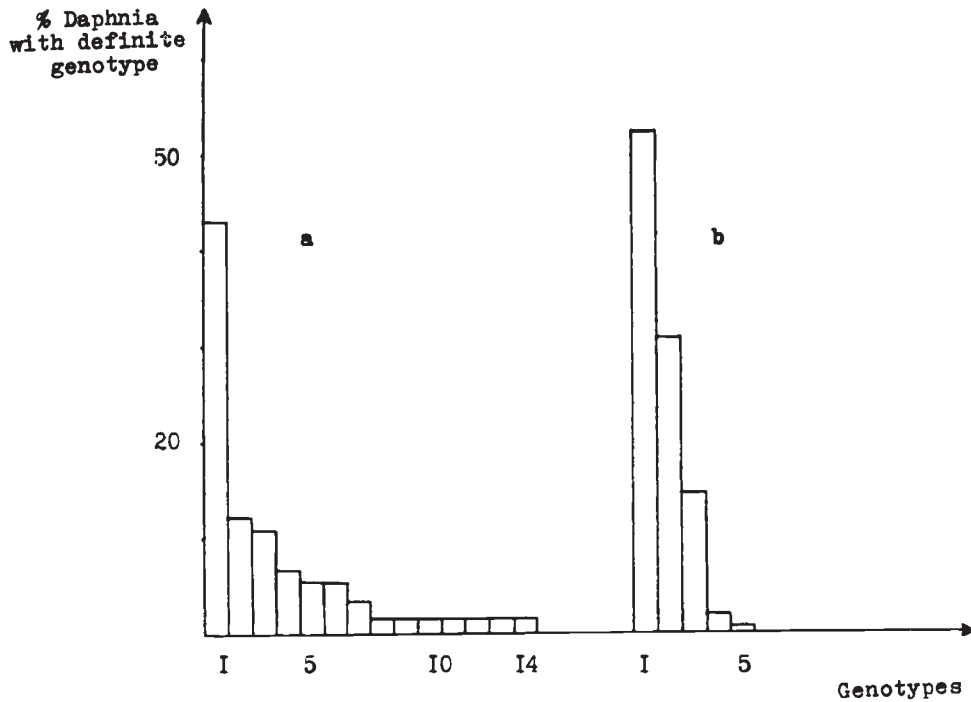


Figure 3 Changes in the genotypic constitution of population Z at the beginning (a spring, sample size is 75 individuals) and the end (b autumn, sample size is 164 individuals) of the period of parthenogenetic reproduction. Estimation of the genotypic constitution was based on variations in esterase genotypes.

DISCUSSION

Integration of parthenogenesis and bisexuality and the transition between them is an outstanding evolutionary feature of *Daphnia pulex*, of other species of Cladocera, as well as of some Rotifera and Nematoda (White, 1977). The emergence and maintenance of this unique reproductive system was probably due to the requisite for successful occupation of habitats with sudden changes in conditions. The origin of the system will not be considered here; an attempt will be made to examine some of the factors that maintain its stability.

It is recognised that one of the components of

the bipartite system, parthenogenesis, confers with short-term advantages. One advantage is two-fold: it makes males unnecessary and concurrently decreases segregational losses (Maynard Smith, 1978). Parthenogenesis is also advantageous because it increases the efficiency of natural selection: clones less endowed with the capacity for active parthenogenetic reproduction are eventually eliminated. A detailed proof has been presented elsewhere (Ruvinsky *et al.*, 1985).

The occurrence of bisexuality in natural populations, in spite of the undeniable advantages of parthenogenesis, returns us to the perennial problem of the short-term advantage of the bisexual mode of reproduction (Maynard Smith, 1978).

Table 2 Genotypic and sexual structure of the *Daphnia pulex* population Z during bisexual reproduction (October 1981)

Genotype	Females %		Males %
	Ephippial	Non-reproducing	
<i>Est</i> - 1 ^{b,b} <i>Est</i> - 2 ^{b,b} <i>Est</i> - 3 ^{a,a(a,o)}	23	27	44
<i>Est</i> - 1 ^{a,b} <i>Est</i> - 2 ^{b,c} <i>Est</i> - 3 ^{a,a(a,o)}	57	63	33
<i>Est</i> - 1 ^{a,a} <i>Est</i> - 2 ^{b,c} <i>Est</i> - 3 ^{o,o}	18	10	17
<i>Est</i> - 1 ^{b,b} <i>Est</i> - 2 ^{b,b} <i>Est</i> - 3 ^{a,d}	2	0	0
<i>Est</i> - 1 ^{a,a} <i>Est</i> - 2 ^{b,b} <i>Est</i> - 3 ^{a,c}	0	0	6
Studied individuals	56	60	48

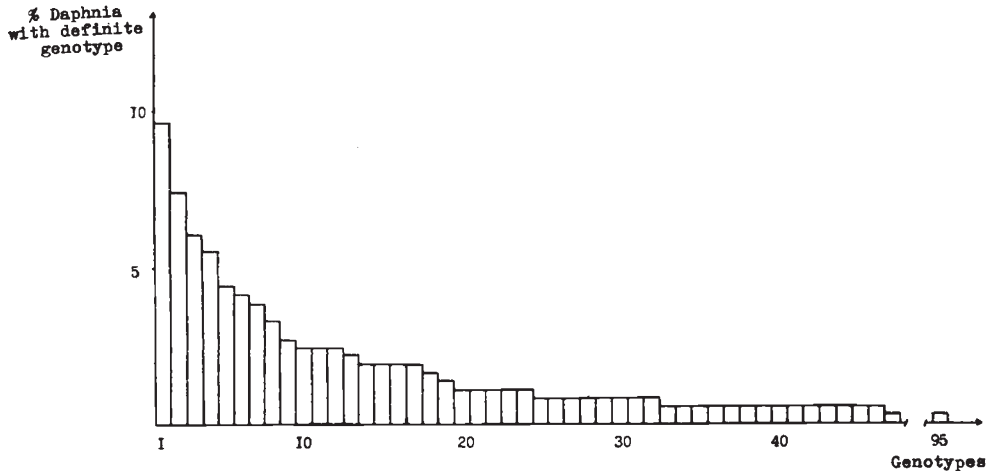


Figure 4 Distribution of the occurrence frequencies of 95 esterase genotypes detected in samples of *Daphnia* from population Ch in 1982. Sample size is 364 individuals.

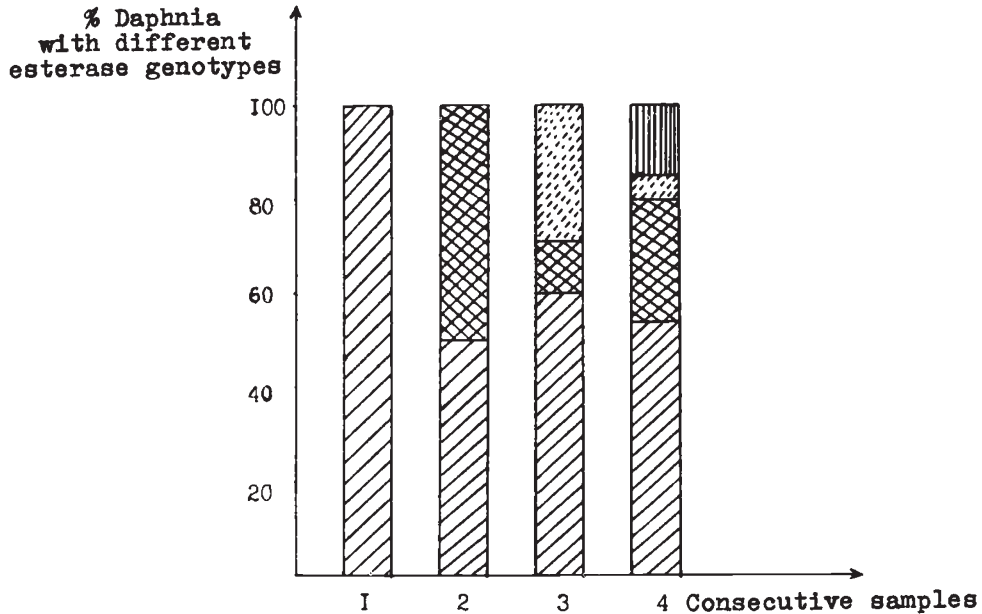


Figure 5 Genotypic constitution of population Ch. in four consecutive samples obtained in 1982. Esterase genotypes first detected I. ▨ (82 individuals), II. ▩ (88 individuals), III. ▧ (92 individuals), IV. ▣ (102 individuals). The number of esterase genotypes detected in sample I is 32 (50-60 per cent of *Daphnia* have these genotypes in the consecutive samples), and that in sample II is 41; of these, 26 are not found in the preceding sample (the percentage of *Daphnia* with the latter genotypes decreases to 10-25 per cent in the consecutive samples). The number of esterase genotypes identified in sample III is also 41; of these, 22 are not found previously, and the percentage of *Daphnia* with these genotypes in the sample is 30 per cent, and it is only 5 per cent in the next sample. Of the 43 esterase genotypes identified in sample IV, 15 is not found previously, 13 per cent of *Daphnia* have these esterase genotypes in the sample.

Bisexuality does endow with short-term superiority. To illustrate, clone ZS-9, which had easy recourse to bisexual reproduction, was more resistant to crowding effects and, consequently, it returned more readily to parthenogenesis when the size of the population decreased. Thus short-term

advantage presumably prevented its exclusion by competitor clone ZS-5. Thus, the positive correlation between resistance to crowding and the capacity for bisexuality, in conjunction with density-dependent selection, may save clones strongly tending to bisexuality from competitive exclusion.

Taken together, all these selective factors favour clonal co-existence in natural populations (Maynard Smith, 1978) and preserve their potential capacity for bisexuality during parthenogenesis. (Hebert, 1978, Young, 1979, Banta, 1939, Maynard Smith, 1978). Consequently, regular increase in population density provides a means for selecting clones concomitantly capable of parthenogenetic and bisexual reproduction.

The described clonal co-existence in a parthenogenetically reproducing population serves to illustrate the more general treated principle of Gause (Gause, 1934). With reference to the present case, it may be stated that the larger is the number of clones in a population, the better the population takes advantage of its ecological niche, the more it resists vicissitudes, and the less it varies in size. Support for these statements probably derives from Brookfield (1981).

Young's paper (Young, 1979) may be also implicitly relevant to our understanding of the mechanism providing clonal co-existence. In the *Daphnia* population he examined, genotype GOT-FF prevailed at increasing population densities and genotype GOT-SF at decreasing ones. His observation may be explained in terms of proneness to a particular mode of reproduction: parthenogenetic for GOT-FF and bisexual for GOT-SF clones.

Besides short-term mechanisms maintaining polymorphism with respect to reproduction mode, there is a long-term one dependent on annual cycles. Because bisexuality is the only way of transmitting genetic information from one year to the next, clones lacking this capacity contribute poorly, if at all, to the genetic pool next year, although the numbers of these clones can be vast in autumn. Clearly, these clones are eliminated.

One might expect, at first sight, that narrowing of clonal diversity during parthenogenesis and incapacity for bisexuality would deplete genetic variation, produce greater homozygosity owing to increased inbreeding ultimately resulting in loss of evolutionary flexibility. There are, however, mechanisms counteracting these events. One possible mechanism is the disconnection of physiological processes giving rise to males and ephippial eggs. It makes more probable the occurrence of matings between different clones. Another possible counteracting mechanism may be low mating efficiency within clones (Ruvinsky *et al.*, 1985).

Genetic diversity, as well as the parthenogenesis to bisexuality relationship, are subject to adjustment to changing environments. This is apparent when comparing populations liv-

ing in extremely different conditions. The consequences of life in fairly constant conditions are longer periods of parthenogenesis (5-6 months), narrowed genotypic diversity (5-6 esterase genotypes), increase in the number of predominantly parthenogenetic clones (population Z, fig. 3). The consequences of life in extremely variable conditions are shortened to minimum periods of parthenogenesis (4-5 weeks), with highly diversified genotypes and strong tendency to bisexuality (population Ch. fig. 4).

The fluctuating balance established between parthenogenesis and bisexuality, as well as coping by genetic polymorphism with ecological demands, provide high adaptability of populations. Natural populations of *Daphnia* are under the impact of environments with often unpredictable adversities. This calls for large genetic variations in many systems, including reproduction. The resultant labile polymorphism seems to rely on the switching over from one developmental programme to another. We have referred to this variability as alternational and caused by activation of some genetic systems and inactivation of others (Ruvinsky *et al.*, 1978, 1983a, b). According to Schmalhausen (1949) such systems can be established under the effect of stabilizing selection. The alternational polymorphism is of a regulatory kind and, in a sense, dependent on genetic diversity. This may be illustrated by the variations in the capacity for reproduction mode (table 1) and the variations in the electrophoretic mobility of the glucose-6-phosphate dehydrogenase in *Daphnia pulex* clones (Ruvinsky *et al.*, 1983a, b, 1986).

With regard to maintenance of polymorphic systems in *Daphnia* and other species with a similar mode of reproduction, a scheme is suggested (fig. 6). The high adaptivity of cyclic parthenogenesis and its non-ubiquity raise the question of why this efficient device has found limited application during evolution. A possible explanation may be that cyclic parthenogenesis of cladoceran type becomes advantageous only when the length of the life cycle of an individual keeps up with the rate of changes in ecological conditions. Therefore, taxons with larger life cycles lacking this temporal correspondence observed during cyclic parthenogenesis of cladoceran type are waste efforts.

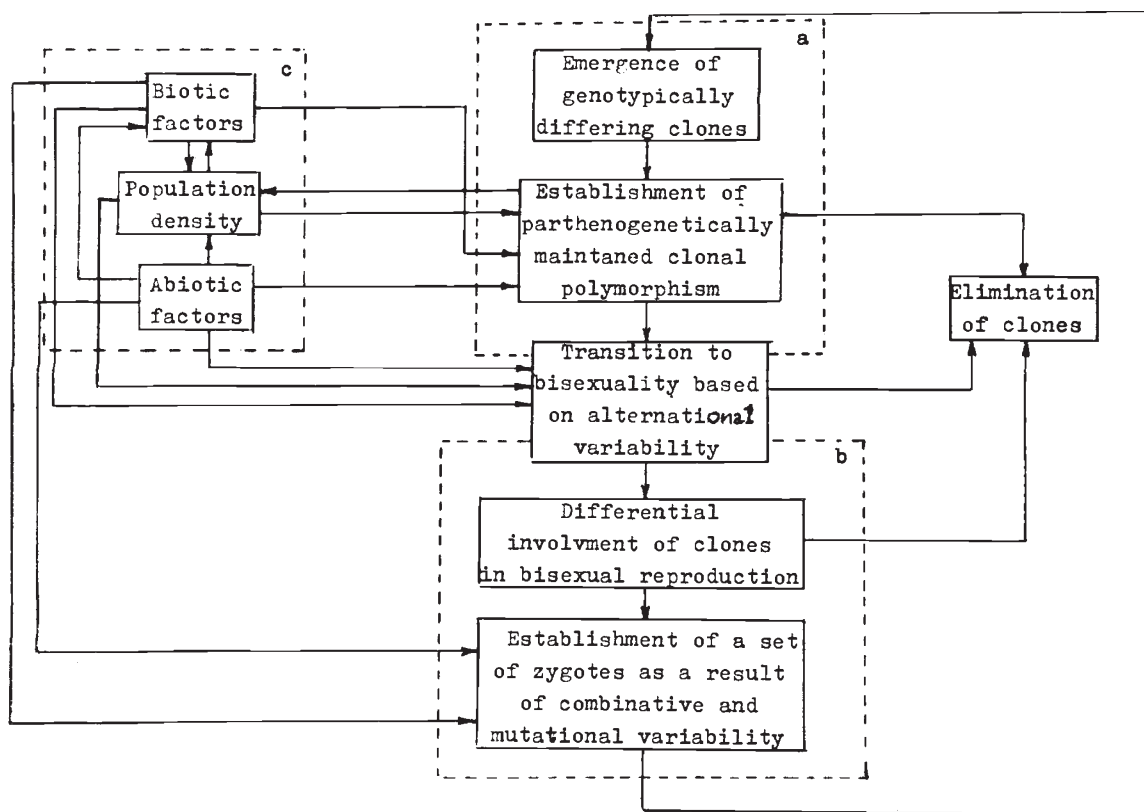


Figure 6 A scheme suggested for the organisation and maintenance of polymorphism in *Daphnia pulex* populations. Reproduction mode: (a) parthenogenetic, (b) bisexual, (c) environmental factors.

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