Enzymatic variability of a colonizing population of *Daphnia obtusa* Kurz (Crustacea, Cladocera) in Lake Orta (Italy)

A. Bachiorri, V. Rossi, C. Bonacina and P. Menozzi

Introduction

The study of enzyme variability by electrophoresis in *Cladocera* is by now a field with an established tradition (Herbert 1974a, b, Innes et al. 1986, Hann & Herbert 1986, Herbert & Crease 1980). The clonal structure of these parthenogenetic organisms (cyclical or obligate) has been investigated and ecological differences in requirements of different clones have been reported (Weider & Lampert 1985, Weider & Herbert 1987).

Extreme environmental factors may select clones with substantial differences in life-history traits and in the ability to colonize new habitats (Loaring & Herbert 1981, Weider & Lampert 1985, Weider & Herbert 1987). *Daphnia obtusa* Kurz (Crustacea, Cladocera) usually lives in small water bodies such as temporary ponds. This species recently colonized (first found in September 1986) Lake Orta, a large, severely polluted lake in Northern Italy. Heavy metals (Cu, Ni, Zn, Cr) and acid pollution (pH values of 3.8-4.3 at the overturn) had almost completely destroyed any life forms in the lake for several decades (Mosello et al. 1986, Bonacina et al. 1988).

Here we report an eighteen month study of the genetic structure of the *Daphnia obtusa* population that colonized Lake Orta. A comparison with nine populations from other localities is also reported.

Materials and methods

To evaluate temporal and spatial distribution of *Daphnia obtusa*, sampling was performed monthly from November 1987 to May 1989, in three different locations in Lake Orta (Fig. 1).

For comparison, *Daphnia obtusa* populations from 8 small lakes or temporary ponds and from a laboratory strain were also analysed (Fig. 1).

Animals were taken to the laboratory and kept alive in filtered Lake Maggiore water. Electrophoresis was performed within 48h in 11% starch gels; procedures and staining methods were described in Wolf (1982) and Shaw & Prasad (1970). The small size (0.7-2.2 mm) of the animals allowed the simultaneous testing of no more than 4 enzymes. Twenty-seven enzymatic systems were analysed: 6PGD, GOT, GPI, MPI, APH, PGD, ACPH, MDH, ME, ES, ICD, SORDH, ALD, AO, LDH, HK, LAP, XDH, HBDH, Gd, GAPDH, GDH, GLDH, GPD, SOD, ODH and cis-Acon.

In the laboratory, clones were obtained from a single female randomly selected from samples taken at different times and sites in Lake Orta (clone LO) and were also established for the other sources of *Daphnia obtusa*.

Clones were reared in filtered, aerated Lake Maggiore surficial water, at 20±1°C, under a 12 h-dark/12 h-light period and fed a suspension of *Scenedesmus obliquus* (exponential growth phase).

Electrophoretic analysis was repeated on each clone for up to 12 generations to test the stability of electrophoretic morphs.

For sufficiently polymorphic loci (rarest genotype > 1), genotype frequencies were tested for fit to Hardy-Weinberg expectations. Gene frequencies were determined by gene counting. Average heterozygosity H (for each locus, H = 2p1p2, where p1 and p2 are allele frequencies when in H-W equilibrium, H = frequency of heterozygotes otherwise) and genotypic diversity

0368-0770/91/0024-2813 $ 0.75
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Fig. 2. Electrophoretic morphs for enzymes that showed variability. Allelism was determined assuming a monomorphic quaternary structure for all loci.

G₀ (G₀ = 1/Σgᵢ², where gᵢ is the relative frequency of the iᵗʰ multilocus genotype) were calculated for each locality.

To evaluate differences in allele frequencies among populations, G-statistics were calculated.

Nst's genetic distance D (D = -ln(Σ Xᵢ Yᵢ / Xᵢ Yᵢ + Yᵢ Xᵢ), where Xᵢ is the relative frequency of allele i in the X population and Yᵢ is the relative frequency of allele i in the Y population) and principal component analysis were used to compare genetic structure and geographic location.

Results and discussion

The analysis of the twenty-seven enzymatic systems revealed activity for the following 15 enzymes: 6PGD, GOT, GPI, MPI, APH, PGM, ACPH, MDH, ME, ES, ICD, SORDH, ALD, AO and LDH. The electromorphs tested in laboratory maintained clones were stable for up to 12 generations.

6PGD, AO, GPI, MDH, ICD, ACPH, LAP, ME, SORDH, ALD, LDH were homozygous in all populations studied. Two loci were determined for GOT and ES. Under our laboratory procedures, GOT showed two morphs migrating anodally and cathodally, respectively. The ES electromorphs were interpreted as two loci following the literature (HEBERT & CREASE 1983).

The Lake Orta population is made up by only one multilocus genotype: the same electromorphs were observed for all the 1341 individuals studied (Fig. 2). No change in time or space was observed.

In spite of the much smaller sample sizes, the other 8 populations did show some variability (Table 1). Significant differences in gene frequencies among populations exist for all loci with the exception of PGM (Table 1).

Given the presence of only one multilocus genotype in Lake Orta and the high number of monomorphic loci, the analysis of H-W equilibrium is of limited interest. Not surprisingly, the genetic diversity is low for all populations if compared with data from Canada (INNES et al. 1987). Smaller sample sizes (with the exception of the Lake Orta sample) may in part be an explanation for such differences. No clustering exists in the plot of the first two principal component on gene frequencies.

Table 1. Allele frequencies, average heterozygosity (H) calculated over all 17 loci, sample size (N), genotypic diversity (G₀) and number of genotypes (Nₓ) are indicated. G-statistics values are reported and * indicates significant differences (p < 0.05) among populations.

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Fig. 3. Plot of first and second principal components of *D. obtusa* populations based on seven-locus genotypes. The first two components explain 70% of the total variation.

(Fig. 3). As expected, there is no relationship between geographic and genetic distance. Although males were sampled in the Lake Orta, the observed genetic homogeneity is evidence of their lack of function. No ephippial females were observed in Lake Orta. The only other population sampled at two different dates (Cabriolo, Fig. 1) also showed no changes in gene frequencies.

In Lake Orta, *D. obtusa* shows an obligate parthenogenetic mode of reproduction. For the other populations, sampling in time will be necessary for firmer conclusions.

In the Lake Orta population, there is clearly a strong selection in favour of the multilocus genotype that colonized this environment. The ability of *D. obtusa* to colonize stressed habitats (Fryer 1985) is thus confirmed.

The "liming experiment" in progress during the summer of 1989 will offer an interesting opportunity to validate this conclusion.

References


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