Large Hardy-Weinberg equilibrium deviations in the *Daphnia longispina* of Lake El Tobar

Charles E. King¹, Maria R. Miracle² & Eduardo Vicente²

¹Department of Zoology, Oregon State University, Corvallis, Oregon 97331, USA
²Department of Ecology, Faculty of Biological Sciences, University of Valencia, 46100 Burjasot (Valencia), Spain

Key words: *Daphnia*, allozymes, Hardy-Weinberg equilibrium, parthenogenesis

Abstract

The population structure of *Daphnia longispina* in Lake El Tobar, Spain was studied by measuring variation at the aldehyde oxidase (AO), phosphoglucose isomerase (PGI) and phosphoglucose mutase (PGM) loci in each of 1337 individuals from four collections. In 9 of the 12 comparisons between observed allele frequencies and those expected by Hardy-Weinberg equilibrium there was an excess of heterozygotes. We found 27 of the potential number of 54 composite electromorphs (‘clones’) based on the three allozymes. Clone diversities were rather high in all collections. Three clones reached frequencies of over 25% and different clones were dominant in each of the four collections. Strong temporal variation was found in the genetic structure of this *Daphnia* population. This variation was driven by changes in the relative frequencies of the component clones in the lake rather than by a recruitment of novel clones into the population.

We conclude with a consideration of the role of models relating allele and genotype frequencies in populations of cyclical parthenogens. Because the breeding system of these populations infrequently involves recombination between clones, models such as the Hardy-Weinberg have limited value in providing meaningful measures of population structure.

Introduction

*Daphnia* populations may be viewed as dynamic assemblages of interacting clones. In comparison with most other zooplankton taxa, their genetic structure and the role of natural selection acting on *Daphnia* in different types of habitats such as intermittent ponds, permanent ponds and lakes has been extensively studied (e.g., Weider, 1985; Hebert, 1987; Lynch, 1987; Wolf, 1988). The importance of interspecific hybridization and the possibility of genetic transfers between cyclic parthenogens and obligate asexual clones are now well documented in *Daphnia* (e.g., Wolf & Mort, 1986; Hebert *et al.*, 1988, 1989; Crease *et al.*, 1989; Taylor & Hebert, 1992, 1993; Weider, 1993; Spaak, 1994). However, it is still unclear whether and under what circumstances a seasonal succession of genetically distinct clones takes place.

In this paper we demonstrate that both allele and clone frequencies of *Daphnia longispina* in Lake El Tobar (Spain) show temporal variation from one collection period to the next. The isolation of this lake precludes a high frequency of exchanges between its population and those from other localities. While the causes of these temporal changes are difficult to assess they clearly lead to departures from Hardy-Weinberg equilibrium.

Materials and methods

Field methods

El Tobar is a karstic lake in the Cuenca mountains of Spain (UTM 30TWK806888) at an altitude of 1250 m. It consists of two main basins. The large basin (57 Ha, 12 m maximum depth) is an elongated limestone sink,
fed mainly by underwater springs located along its eastern shore. The small basin (10 Ha, 19.5 m maximum depth) is located in an indentation of the northern shore. Its conductivity is about 0.6 mS cm\(^{-1}\) in the mixolimnion from the surface to the chemolimnion which is at a depth of about 12 m. Below the mixolimnion, conductivity drastically changes to reach almost 200 mS cm\(^{-1}\) at the bottom. The small basin lies at the base of a steep cliff, is more sheltered from the wind than the main basin and, because it protrudes laterally, is more isolated from the main water flow (NE-SW). More information on the morphometry, hydrology, chemistry and planktonic populations of this lake can be found in Vicente et al. (1993) and Miracle et al. (1993).

All samples were taken in the center of the small basin using ropes attached to trees and rocks on the shore to fix the position of the collecting boat. Vertical profiles of temperature, conductivity, oxygen and light penetration were measured in situ with the appropriate sensors (WTW and Li-Cor instruments). Water samples for chemical analyses and phytoplankton were taken with a Ruttner bottle. Zooplankton was filtered in situ from water samples taken with a double Van Dorn bottle (5.41 capacity and 35 \(\mu\)m mesh) and a Patalas trap (25 l capacity and 100 \(\mu\)m mesh).

Collections were made on four occasions: August 11, September 21–23, November 19, 1991 and April 22, 1992. For the last three sampling dates, day and night 1 m-interval profiles of zooplankton were taken. Formalin preserved samples were counted and the numbers of eggs per female counted. In September, when vertical heterogeneity was highest, four profiles, from different times of the day, were examined.

Genetic analysis of Daphnia was based on live samples taken by a net tow at the surface and with Van Dorn or Patalas traps at 5 and 10 m depths at noon and midnight of the above mentioned dates, except for August from which only noon samples from surface and 11 m were analyzed. Small supplementary samples from 5 and 9 m depths were also made in August to screen for electrophoretic variation. In September four collections were made on successive days, two at noon and two at midnight. Immediately after collection, in a caravan on the shore of the lake, adult individuals from each of the three depths were isolated and placed singly in 0.5 ml eppendorf tubes with 5 \(\mu\)l of tris-glycine tray buffer. They were then frozen on dry ice and transported to the laboratory in Valencia where they were stored at \(-80^\circ\)C.

**Electrophoresis**

Isozymes were detected by means of cellulose acetate electrophoresis (Hebert & Payne, 1985) of whole-animal homogenates using single daphnids. Individuals from several depths (surface net tow, 5, 9 and 11 m) of the August collection were screened for 13 enzymes. No variation was found in MDH (malate dehydrogenase, E.C. 1.1.1.37), APK (arginine phosphokinase, E.C. 2.7.3.3), IDH (isocitrate dehydrogenase, E.C. 1.1.1.42), FUM (fumarate hydratase, E.C. 4.2.1.2), 6PGDH (6-phosphogluconate dehydrogenase, E.C. 1.1.1.44) or GOT (glutamate oxaloacetate transaminase, E.C. 2.6.1.2). Variation was present in LDH (lactate dehydrogenase, E.C. 1.1.1.27), G6PDH (glucose-6-phosphate dehydrogenase, E.C. 1.1.1.49), MPI (mannose phosphate isomerase, E.C. 5.3.1.8), and ME (malic enzyme, E.C. 1.1.1.40); however these enzymes were not selected for further analysis because of technical limitations.

Three additional enzymes were found to be highly variable in the El Tobar samples, had strong activity with consistently good resolution, and produced readily interpretable patterns. Each of the individuals in this study was scored for genotype at these three loci. AO (aldehyde oxidase, E.C. 1.2.3.1) and PGI (phosphoglucone isomerase, E.C. 5.3.1.9) are dimeric enzymes and each had two alleles at a single locus. PGM (phosphoglucomutase, E.C. 5.4.2.2) is a monomer and had three alleles at a single locus. These three enzymes were used to score 78 individuals from the August collections, 600 from September, 341 from November and 258 from April.

Groups of phenotypically identical individuals based on the three enzymes we assayed are most precisely designated as either ‘composite electromorphs’ or ‘multilocus genotypes’ since these terms make no presumption regarding the variation— or lack of variation— present at other loci. The scoring of additional loci might well reveal that one or more of the different composite electromorphs could be further subdivided into distinct groups. This potential exists for any electrophoretic study of field populations. With this qualification in mind and for brevity and as a matter of convenience, the different composite electromorphs will be referred to herein as different ‘clones’.