# Genetic Diversity of *Daphnia* in Alpine Lakes

**The diversity of zoo- and phytoplankton in alpine lakes has been studied intensively for over 100 years. It is now generally accepted that the species diversity of plankton communities decreases with increasing altitude. This study addresses the question as to whether or not this general pattern also applies at the population level. *Daphnia*, a typical member of zooplankton communities, was chosen as the study organism. Genetic diversity was determined for 11 *Daphnia* populations in mountain lakes at varying elevations. We found that the genetic diversity was very heterogeneous.**

The extreme environmental conditions in alpine regions result in a small selection of flora and fauna that can adapt to survival in these regions; therefore, biodiversity (or more precisely species diversity), decreases with increasing altitude. It is unclear, however, whether or not this is also reflected in another aspect of biodiversity; namely, genetic diversity. In order to answer this question, we examined *Daphnia* populations, a typical member of zooplankton communities, in a number of Swiss mountain lakes. These organisms are particularly interesting for such a study since they can reproduce sexually or asexually by parthenogenesis [1].

## Parthenogenesis and Resting Eggs

Under favorable environmental conditions, parthenogenesis is the normal reproductive method in *Daphnia*; mother organisms form genetically identical daughters (so-called clones). This allows *Daphnia* to multiply extremely rapidly, thereby making optimal use of short growth periods in alpine ecosystems. When environmental conditions worsen, e.g. during cold periods or when food is sparse, *Daphnia* produce males and sexual females. Sexually fertilized eggs are encased in a resistant shell, the so-called ephippium, which resides in the breeding cavity and is sloughed with the next shedding of the skin. Ephippia can survive for years in the lake sediments. Under favorable conditions, these resting eggs can produce parthenogenetic females, and the cycle begins again. If the conditions are favorable all year, no resting eggs are formed, so that one parthenogenetic generation follows another.

## Environment, Reproduction and Genetic Diversity

Our project investigated the relationship between environmental conditions, reproduction method, and genetic diversity in alpine lakes. We examined 11 mountain lakes in the Swiss Alps. The lakes varied in elevation, where environmental conditions become harsher as elevation increases. *Daphnia* populations in these lakes were sampled in four consecutive years (1997–2000) during late summer or early fall.

Our experiments were based on the following hypotheses:

- Alpine lakes should harbor a high proportion of sexual individuals, since *Daphnia* can survive cold and food-deprived winters only in the form of resting eggs.
- In *Daphnia*, genetic diversity depends primarily on the sexual exchange of genetic material. We postulated that populations in lakes, where *Daphnia* reproduce mostly sexually, would exhibit higher genetic diversity.
- Since environmental conditions in alpine lakes become harsher with increasing elevation, we expected that the genetic diversity in *Daphnia* populations would increase with increasing altitude and not decrease as is the case for species diversity. Alpine lakes should, therefore, show higher genetic diversity than lakes in the lowlands.

### The Genetic Examination Method

**Allozyme electrophoresis is a proven method for the determination of genetic diversity of populations [2]. It is based on the following principle: the genome of an organism may contain the genetic information for a certain enzyme once or several times. If the gene is present several times, it is likely that the individual genes have changed over time, usually due to mutations. As a result, the cell will synthesize different enzymes, so-called allozymes, which have slight differences in their amino acid sequences, but will exhibit the same functionality as the original enzyme. In the simplest case, there are two allozymes which differ by only one amino acid. If the new amino acid carries a different charge, the two allozymes will have different migration velocities in an electric field and can be separated by gel electrophoresis. Subsequently, the enzyme can be detected through the reaction with its substrate. If the substrate reaction is coupled with a color reaction, the enzyme can be made directly visible. This method indicates how many enzyme variations are present within a population and, therefore, provides an index of genetic diversity.**

## Sexual Individuals in the Majority

The relative fraction of asexual and sexual *Daphnia* in our study lakes varied dramatically and ranged from 2 to 90% (Fig. 1 lower panel). In 8 of the 11 mountain lakes examined, sexual *Daphnia* outnumbered asexual individuals. In the two lowest alpine lakes, the Upper and the Lower Arosasee, we found almost exclusively asexual females, while the highest lake, Riffelsee II, contained almost exclusively sexual individuals. The distribution between sexual and asexual *Daphnia* for lakes at intermediate elevations, however, was heterogeneous; we could not decipher any clear patterns.

There was, however, one surprising finding: while males often made up less than 1% of the *Daphnia* populations in lowland lakes, they made up as much as 30–40% of the populations in the mountain lakes. In addition, the fraction of sexual male individuals in alpine lakes was always higher than that of sexual females.

## Genetic Diversity

In order to assess the genetic diversity of different *Daphnia* populations, we examined up to 80 individuals per lake by the allozyme method (see box). The clonal diversity, i.e., the number of different clones within a population, is a first indication of overall diversity. In the 11 mountain lakes examined in this study, we found between 2 and 42 different clones (Fig. 1 upper panel). Melchsee and Lago Cadagno showed the highest clonal diversity.

A better description of genetic diversity is obtained when the number of individuals for each of the clones is taken into account in addition to the number of different clones. A population with 10 different clones, each making up 10% of the population, is more diverse than a population with 10 different clones, but one single clone accounting for 99% of all the individuals. Therefore, we calculated the Simpson index ($D_{Sim} = 1 - \sum p_i^2$, $p_i$ = fraction of a clone i in the overall population) for each of the 11 populations that were examined (Fig. 1 upper panel). This index describes the probability that two randomly chosen individuals have a different genetic structure. High $D_{Sim}$ values (near 1) indicate that all clones occur with similar frequency, which translates to high diversity. Low $D_{Sim}$ values (near 0) show that one clone dominates the population, resulting in low diversity. It was found that the diversity of *Daphnia* populations was relatively high in a number of lakes under examination, whereas it was low in the Upper Arosasee, Leisee and Riffelsee II.

## The Spatial Genetic Structure of *Daphnia* Populations is Complex

The high proportion of sexual individuals in alpine lakes was surprising and is an indication that *Daphnia* survive the harsh winters only in the form of resting eggs; however, the fraction of sexual individuals did not correlate with genetic diversity, neither if expressed as clonal diversity nor in the Simpson index. Furthermore, we were not able to establish a correlation between the genetic diversity of *Daphnia* populations and the elevation of the alpine lakes examined in this study. We compared our data to the genetic diversity of *Daphnia* populations in two lowland lakes, namely Greifensee ($D_{Sim}$ = 0.96) and Lake Lucerne ($D_{Sim}$ = 0.48). We found that the genetic diversity in mountain lakes can be as low as that of Lake Lucerne, or almost as high as that of Greifensee.

This study [3] represents a first step in examining the genetic diversity of a major zooplankton species in alpine lakes and in determining those factors that influence genetic diversity. Apparently, sexual reproduction does not necessarily lead to higher genetic diversity in *Daphnia* populations. It can, therefore, be assumed that other factors, such as the presence of certain *Daphnia* species or *Daphnia* hybrids, have a stronger influence or that only certain clones are adapted to the extreme environmental conditions in alpine lakes. High genetic diversity does not appear to be a prerequisite for survival of *Daphnia* in alpine lakes. Other strategies, such as their flexible behavior and life cycle, may be more important in coping with the harsh conditions in the alpine environment.

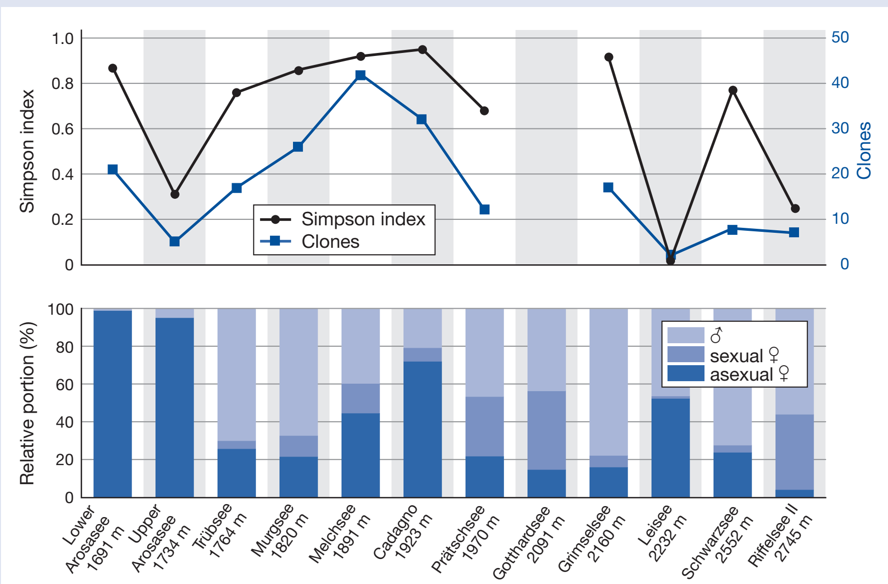


**Fig. 1: Investigation of *Daphnia* populations in 11 Swiss alpine lakes at varying elevations. Lower panel: relative proportions of sexual and asexual individuals; upper panel: genetic diversity, expressed as number of clones and by the Simpson index.**

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