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## RESEARCH PROJECT

### Genetic architecture of traits associated with habitat adaptation in *Silene vulgaris* (Caryophyllaceae)

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#### Summary

1 Understanding the mechanisms underlying speciation is a key problem of evolutionary biology. Studies on the genetic basis of adaptation to particular site factors and the role of these adaptations in plant speciation are fundamental to our understanding of the origin of plant biodiversity. In this project, we study the genetic basis of adaptation to serpentine soil as an incipient step in habitat-driven speciation in the bladder campion, *Silene vulgaris*.

2 Previous studies have shown that *Silene vulgaris* plants growing on serpentine soil near Davos (CH) and plants growing in neighbouring non-serpentine areas are morphologically distinct and differ largely in tolerance to high nickel concentration. We will carry out pollination experiments to test whether reproductive isolation has already evolved between neighbouring serpentine-adapted and non-adapted populations in Davos and if so, if barriers are genetically determined or habitat-induced.

3 The genetic architecture of adaptation to the serpentine habitat will be studied by constructing a linkage map in an F2 population resulting from a cross between a tolerant and a non-tolerant plant. Mapped molecular markers will then be correlated with morphological traits of plants and with physiological tolerance to high Ni concentration and low Ca/Mg ratio to determine quantitative trait loci (QTL) for adaptation to serpentine soil.

4 To study the relevance of particular QTL for plant performance under natural conditions, cuttings of plants used in genetic mapping will be transplanted into the field at sites with serpentine and non-serpentine soils. The performance of transplants will be monitored during two growing seasons.

5 This combination of genetic mapping with physiological studies and tests of the ecological performance of the same plants under natural conditions will make it possible to study relationships between adaptation and fitness at a much finer scale than population-level studies. The results of our project should therefore considerably increase our understanding of the genetic basis of adaptation and, eventually, of speciation in plants.

**Keywords:** heavy-metal tolerance, QTL, reciprocal transplant experiment, reproductive isolation, serpentine, speciation

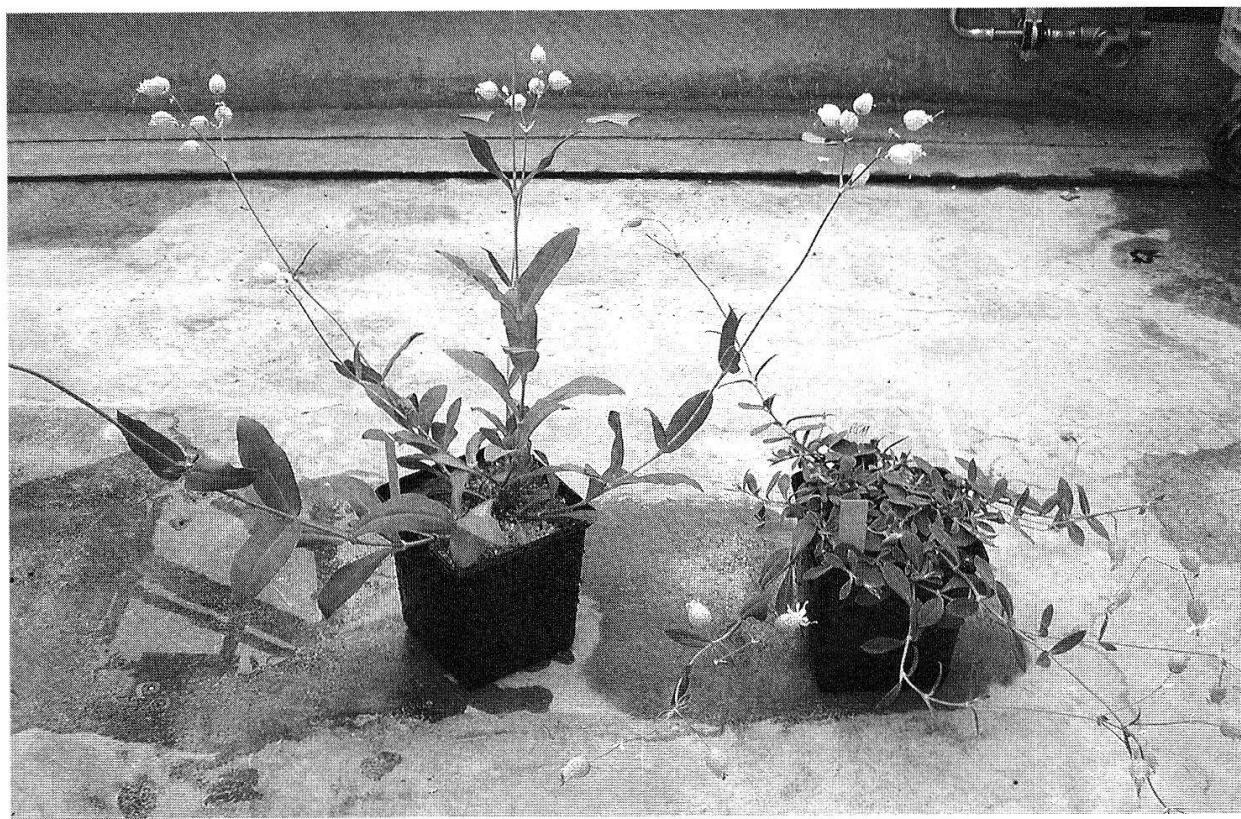
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## Introduction

Adaptation to different habitats may be particularly important in plant speciation because plants are stationary. Any type of habitat subdivision is thus likely to reduce gene flow between populations adapted to different habitats (e.g. Rieseberg *et al.* 1998). Increasing empirical evidence for the sympatric origin of many species lends credit to the idea of sympatric speciation. A well-documented example of small-scale evolution and an exemplary case-study of speciation in action is heavy-metal tolerance in higher plants (Antonovics *et al.* 1971; Proctor & Woodell 1975; Kruckeberg & Kruckeberg 1990). While high concentrations of heavy metals in soil are toxic to most higher plants, some species have developed ecotypes that resist toxicity much better than non-adapted ecotypes.

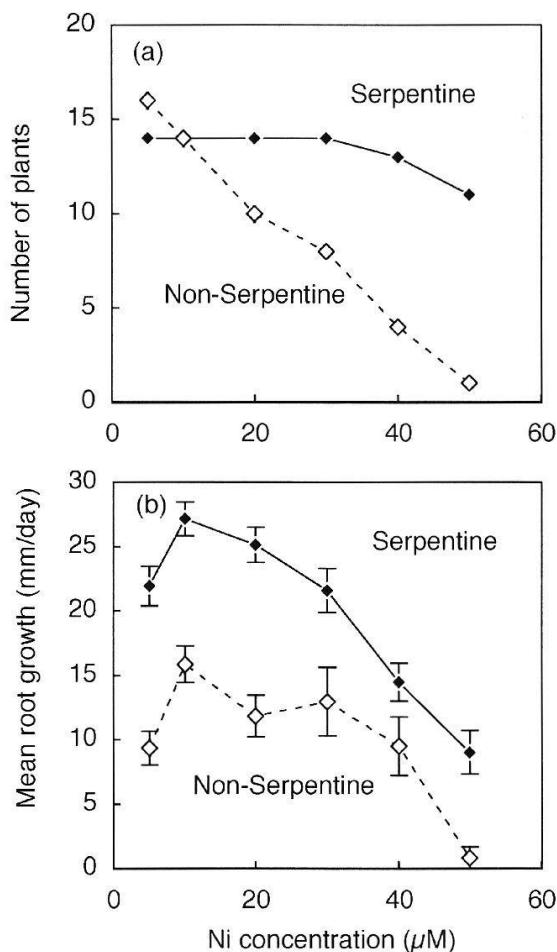
Morphological and physiological adaptations protect the tolerant ecotypes against toxic effects and enable them to maintain a sufficient uptake of essential elements. Studies on the genetic basis of heavy-metal tolerance have suggested that few genes with major effects are involved in tolerance mechanisms (Macnair & Christie 1983b; Schat & ten Bookum 1992). The evolution of these genes may result in reproductive isolation between tolerant and non-tolerant genotypes (Searcy & Mulcahy 1985; Macnair & Cumbes 1989; Searcy & Macnair 1990). In this way, adaptation to heavy metals may eventually lead to sympatric speciation.

Naturally heavy-metal contaminated sites, such as serpentine soils (ultramafic rocks) are found throughout the world (Ernst 1974)



**Fig. 1.** Morphological differences between plants of *Silene vulgaris* cultivated in the greenhouse from seeds collected in a meadow population on fertile soil with normal heavy-metal content (left) and in a serpentine population on 'Totalp' near Davos-Wolfgang (right).

and represent an ideal system to study habitat driven sympatric speciation. In areas that have been little affected by the last glaciation period (e.g. parts of Greece or California), new species adapted to the serpentine habitat, the so-called serpentine-endemics, seem to



**Fig. 2.** Difference in Nickel tolerance between populations of *Silene vulgaris* from serpentine and non-serpentine soil shown with a multiple-concentration test (Schat & ten Bookum 1992). Plants (14 from the serpentine population and 16 from the non-serpentine population) were grown in nutrient solutions with increasing Ni concentrations (two days at each concentration), and their root growth was determined at each concentration by measuring the daily length increment of three randomly selected root segments. When root growth stopped, plants were removed from the experiment to ensure their survival. The figure shows in (a) the number of plants remaining in the experiment (with non-zero root growth) and in (b) the mean daily length increment of roots ( $\pm$  SE) of these plants.

have evolved from non-adapted ancestors (Kruckeberg & Kruckeberg 1990; Macnair & Gardner 1998).

Serpentine soils have several highly disadvantageous properties for plants. They typically have very high contents of heavy metals (particularly nickel, chromium and cobalt), an abnormal ratio of calcium to magnesium, and a poor soil development due to slow weathering of serpentine rock (Proctor & Woodell 1975; Kruckeberg & Kruckeberg 1990; Macnair & Gardner 1998).

One of the largest areas of serpentine soil in Switzerland is situated in the vicinity of Davos (GR). The area is known as the 'Totalp' because of its conspicuously sparse vegetation cover compared to adjacent non-serpentine areas that harbour a rich subalpine to alpine flora (Braun-Blanquet & Rübel 1935; Egger 1994). The nickel concentration in the soil can reach 1.9 %, while 1‰ is considered highly selective for any vegetation (Ernst 1974). Under these extreme conditions, only tolerant genotypes of a few plant species can survive (Egger 1994). The bladder campion, *Silene vulgaris* (Caryophyllaceae), is one characteristic element of this highly specialised flora.

Serpentine populations of *S. vulgaris* on the Totalp show characteristic morphological differences to populations growing in the surrounding area (M. Bratteler and A. Geret, unpublished results). Some of these differences persist when plants are grown in the greenhouse (Fig. 1). Moreover plants grown from seeds collected in serpentine populations are more Ni-tolerant than plants grown from seeds collected in the surrounding areas (Fig. 2): *S. vulgaris* plants from serpentine populations have a faster root growth at all Ni concentrations (Fig. 2b), and their roots continue to grow until much higher Ni concentrations than roots of plants from the surrounding area (Fig. 2a). This demonstrates that the

heavy-metal tolerance of *Silene vulgaris* in Davos is genetically determined and not simply due to phenotypic plasticity.

The present research project will investigate the genetic basis of adaptation to heavy metals in *Silene vulgaris* through a combination of molecular genetics and ecological experiments. The genetics of tolerance to several heavy metals has already been investigated in this species (Schat & ten Bookum 1992; Schat *et al.* 1996; Schat & Vooijs 1997), but without the modern tools provided by molecular genetics. The core of our project will therefore be the identification of quantitative trait loci (QTL) for traits associated with adaptation to serpentine. Fitness consequences of particular QTL under natural conditions will be assessed in a field transplant experiment. Our final aim is to understand whether and how adaptation to heavy metals results in speciation in *S. vulgaris* on serpentine soil.

## Research questions and hypotheses

(1) *Are there reproductive barriers between serpentine-adapted and non-adapted populations of Silene vulgaris? If so, are they genetically determined of habitat-induced?*

Reproductive isolation can occur at various stages of the reproductive cycle and may be due to either genetic or phenotypic (habitat-induced) differences between serpentine-adapted and non-adapted plants. We will investigate whether reproductive barriers operate at the stage of seed set and test the two following, non-exclusive hypotheses:

- Seed set of a tolerant genotype is higher after pollination with pollen from an other tolerant genotype than with pollen from a non-tolerant genotype, and vice-versa, even for plants grown under the same conditions (genetically determined barrier).

- Seed set of a plant grown on serpentine soil is higher after pollination with pollen from an other plant grown on serpentine soil than with pollen from a plant grown on normal soil, and vice-versa, even among plants with same genotype (habitat induced barrier).

(2) *What is the number and magnitude of genetic changes involved in the adaptation to serpentine soil in Silene vulgaris?*

We assume that serpentine tolerance is determined additively by several gene loci, so that it is expressed gradually. Stated in a simplified way, this means: the more of the relevant gene loci are expressed in a plant, the more serpentine-tolerant this plant should be. To estimate the number of loci involved in adaptation and the magnitude of their effect, a genetic linkage map will be constructed for a large number of plants expected to express the full range of genetic constitution from minimal to maximal serpentine tolerance. We expect to find associations between genetic constitution and the expression of morphological and physiological adaptations.

(3) *What are the consequences of individual QTL loci for plant fitness under natural conditions in serpentine and non-serpentine soil?*

We hypothesise that plants expressing gene loci associated with traits relevant for serpentine-tolerance (QTL) will perform relatively better after transplantation in serpentine soil.

## Methods

### REPRODUCTIVE BARRIERS BETWEEN SERPENTINE-ADAPTED AND NON-ADAPTED POPULATIONS

Serpentine-adapted plants of *S. vulgaris* will be grown in a greenhouse from seeds collected in the field. Crosses between unrelated

plants will be made to obtain 30 full-sib families. This procedure will reduce potential maternal effects. Five offspring per seed family will then be grown in serpentine soil and in standard potting soil in the greenhouse, respectively (30 families x 2 treatments x 5 replicates = 300 serpentine-adapted mother plants). Flowers will be emasculated to prevent self-pollination.

Additional plants will be raised to serve as pollen donors. As pollen sources we use five unrelated pollen donors from each of three types: (A) non-adapted plants grown in standard potting soil, (B) plants from serpentine habitats grown in serpentine soil, and (C) plants from serpentine habitats grown in standard potting soil. Pollen from the five donor plants of each type will be pooled, so that three different pollen sources will be available. We assume pollen-source plants (A) to be genetically more different from the 300 mother plants than pollen sources (B) and (C).

Each mother plant will have three flowers pollinated with pollen from each of the three sources, respectively. Seeds from these flowers will be collected and counted. The following three outcomes may be found individually or in combination:

- If genetically determined reproductive isolation between serpentine-adapted and non-adapted *S. vulgaris* populations exists, we expect seed set to be reduced in adapted plants that are pollinated with pollen from source A compared to sources B and C.
- If the reproductive barrier is habitat-induced on the maternal side, e.g. a consequence of the heavy-metal concentration in the style, we expect seed set to be lower when pollen from source C (grown in standard soil) is used to pollinate mother plants grown in serpentine soil (compared to mother plants grown in standard soil)

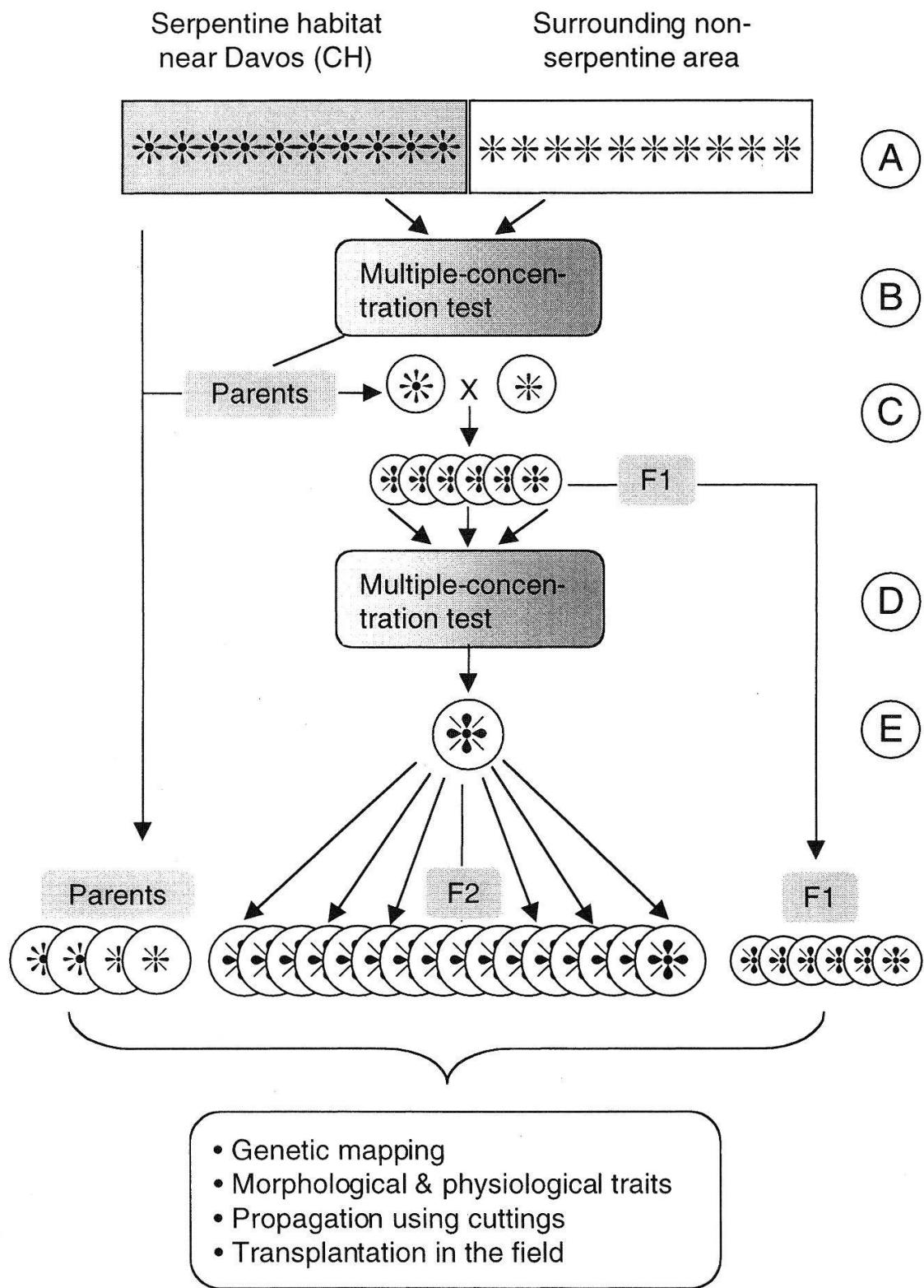
and when pollen from source B (grown in serpentine soil) is used to pollinate mother plants grown in standard soil (compared to mother plants grown in serpentine soil).

- If the reproductive barrier is habitat-induced on the paternal side, e.g. a consequence of pollen quality, seed set should be reduced when mother plants grown in serpentine soil are pollinated with pollen from source C compared to pollen from source B, and when mother plants grown in standard soil are pollinated with pollen from source B compared to pollen from source C.

#### QUANTITATIVE TRAIT LOCI ANALYSIS

Often traits in plants and animals are influenced by many genes rather than by a single locus (reviewed in Falconer 1989). These traits are termed quantitative traits and the loci that control these traits are known as quantitative trait loci (QTL). An important goal in genetics and breeding is to identify and characterize QTLs. The recent advances in molecular biology have allowed the construction of genetic linkage maps based on molecular markers. Such genetic linkage maps can span the genome at regular intervals. The experimenter can then look for correlations between these mapped markers and the trait of interest in controlled breeding experiments to gain insight into the regions of the genome that control the trait.

In our project, a mapping population for QTL analysis was established as represented schematically in Fig. 3: seeds of *Silene vulgaris* were collected in August 2000 in two populations, one from the serpentine area 'Totalp' near Davos Wolfgang, and one from a fertilised meadow near Klosters (< 5 km apart). Twenty seeds from each of ten randomly selected 'source plants' per population were germinated and grown in the green-



**Fig. 3.** Schematic representation of the procedure followed to construct a mapping population of *Silene vulgaris* for the analysis of quantitative trait loci (QTL) for traits associated with serpentine tolerance. The various steps of the procedure were (A) collection of seeds in the field, (B) test of Ni tolerance, (C) crossing of the most and the least tolerant plant, (D) test of Ni tolerance of the F1 offspring, (E) self-fertilisation of the most Ni-tolerant F1 plant and cultivation of the F2 generation, (F) genetic, morphological, physiological investigations and transplantation of cuttings to the field

house of the Botanical Garden of the University of Zürich (Fig. 3A; see Fig. 1). When they were six weeks old, two offspring per source plant were subjected to a multiple-concentration test to identify the most Ni-sensitive and the most Ni-tolerant of the 40 tested plants (Fig. 3B; see Fig. 2). As expected, the most tolerant plant originated from the serpentine habitat, and the most sensitive one from the non-serpentine meadow. These two plants with maximal and minimal Ni tolerance were chosen as parental plants and were crossed experimentally (Fig. 3C). The most tolerant of the F1 offspring (determined with an other multiple-concentration test, Fig. 3D) was self-fertilised to obtain the F2 generation, the mapping population (Fig. 3E). Five hundred F2 progeny are currently grown in a greenhouse and used for the investigations described hereafter (Fig. 3F). Fifty of the F1 offspring and fifty plants of the parental line will be investigated as well.

To construct a genetic linkage map for each of these ca. 600 plants, we will use AFLP, isozyme and microsatellite markers. Mapping will be done in two steps. A fairly detailed initial map (using 250 markers) will be generated for 90 of the 500 F2 progeny. Based on this map, the remaining plants will be genotyped for approximately 120 'framework markers' that are spaced regularly throughout the genome.

The 500 plants of our mapping population as well as the F1 and parental plants are also phenotyped for morphological and physiological traits that may be involved in serpentine adaptation and for heavy-metal tolerance. Morphological traits were measured in spring 2002 in the greenhouse and include approximately thirty characters, such as leaf size, internode distance, stomata density, flower size and time to flowering (number of days between production of the first non-cotyledon leaf and first flowering).

Physiological traits (reaction towards extreme Ca/Mg ratio) and Ni tolerance will be determined in climate chambers with the multiple-concentration test described in Schat and ten Bookum (1992). This method consists in exposing plants to increasing concentrations of a toxic element (e.g. Nickel) or to decreasing Ca/Mg ratios and to measure the growth of selected roots at each of the concentrations after a well-defined period of exposition (as in Fig. 2). The method is non-destructive, as the test ends when root growth stops, while the plant is still living. This makes it possible to quantify the heavy-metal tolerance of individual plants and to use the same plants in further studies, as is needed in our case (question 3).

Associations between genotypes and phenotypes will be analysed with the statistical software QTL cartographer (Basten *et al.* 1997). They will serve to identify the number and location of quantitative trait loci (QTL) for traits involved in serpentine adaptation, and to estimate the magnitude of their effect. Furthermore, the degree of linkage (proximity on chromosomes) and the occurrence of epistasis (non-independence between the effect of one gene and other genes) or pleiotropy (a single gene having multiple effects) among these QTL will be studied.

#### FIELD TRANSPLANTATION EXPERIMENT

According to Antonovics and Primack (1982), field transplant experiments provide a "realistic assessment of genetic and environmental effects". This approach is chosen here to analyse the fitness effects of individual QTL under natural conditions.

All plants included in the genetic mapping study (F2, F1 and parents) are currently propagated using cuttings: shoot sections with two nodes and one leaf pair are replanted in soil; approximately 30% of them

form roots from the nodes and regenerate to a new plant. In spring 2003, two or three cuttings from each plant will be planted in the two habitats of the parental plants (open serpentine soil and fertilised meadow). The performance and fitness of these transplants will be measured as the probability of stem clone survival, early vegetative size (length of the longest leaf), probability of flowering in the first year, total number of flowers produced per plant, seed production, pollen fertility, probability of winter survival, and probability of flowering in the second year.

### Expected results and their relevance

This project combines comprehensive genetic mapping with studies of heavy-metal tolerance and of ecological performance on serpentine soils (field transplantation experiment) using QTL analysis. This approach makes it possible to study relationships between adaptation and fitness with much more detail than population-level comparative studies. The results of our project should therefore increase our understanding of the genetic basis of adaptation to serpentine soils and, eventually, of speciation in plants.

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