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Consequences of ex situ cultivation of plants: Genetic diversity, fitness and adaptation of the monocarpic *Cynoglossum officinale* L. in botanic gardens

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ABSTRACT

Ex situ collections in botanic gardens have great potential in contributing to the conservation of rare plants. However, little is known about the effects of cultivation on the genetic diversity and fitness of garden populations, about genetic changes due to unconscious selection and potential adaptation to the artificial conditions. We compared the genetic variability and fitness of the rare, short-lived perennial *Cynoglossum officinale* from 12 botanic gardens and five natural populations in Germany. Genetic variability was assessed with eight nuclear microsatellites. Plants were grown in a common garden and performance was measured over 2 years. Mean genetic diversity was very similar in botanic garden and natural populations. However, four of the garden populations exhibited no genetic variability at all. Moreover, the genetic diversity of garden populations decreased with increasing duration of cultivation, indicating genetic drift. Plant performance from natural and garden populations in terms of growth, flowering and seed production was similar and in garden populations only seed mass was strongly related to genetic diversity. Several lines of evidence indicated genetic changes in garden populations in response to cultivation. Seed dormancy was strongly reduced in garden populations, and in response to nutrient addition garden plants increased the size of their main inflorescence, while wild plants increased the number of inflorescences. These changes could be maladaptive in nature and reduce the suitability of garden populations as a source for reintroductions. We suggest that botanic gardens should pay more attention to the problem of potential genetic changes in their plant collections.

1. Introduction

Human activities have strongly altered and destroyed natural habitats worldwide and are seriously threatening global biodiversity (Dirzo and Raven, 2003; MEA, 2003; Balmford and Bond, 2005). Strong efforts using a wide range of approaches are necessary to halt this decline (Given, 1994; Krupnick and Kress, 2005). The highest priority is to conserve species in their natural habitats (in situ) where they may experience the full range of interactions with other organisms and where the natural process of evolution can continue. However, to complement in situ efforts to conserve plant biodiversity, the ex situ conservation of threatened plant species in botanic gardens and seed banks is becoming more and more important (Guerrant et al., 2004). The world's botanic gardens not only contain a large collection of plants (more than 80,000 species; Wise Jackson and Sutherland, 2000), but also trained staff and facilities to collect, store and maintain plant material for later reintroduction into the wild (Hurka et al., 2004; Maunder et al., 2004). The Global Strategy for Plant Conservation (CBD, 2002) aims at conserving 60% of all threatened plant taxa in ex situ collections and preparing 10% of them for restoration programmes.

Although a necessary complement to in situ conservation, the cultivation of wild plants in artificial environments is problematic (Hamilton, 1994; Hurka et al., 2004; Husband and Campbell, 2004). Populations of plants cultivated ex situ are usually small, thus exposing them to many of the risks facing small and fragmented natural populations (Schaal and Leverich, 2004). Small and isolated populations are affected by genetic erosion, as genetic variability is increasingly reduced by the combined effects of reduced gene flow and genetic drift, inbreeding increases and deleterious mutations may accumulate (Young et al., 1996; Dudash and Fenster, 2000; Aguilar et al., 2008). Short-lived species are expected to be most strongly affected, as the number of generations in isolation is a crucial factor for the magnitude of genetic erosion in a population (Aguilar et al., 2008). Ultimately, genetic erosion may lead to a decline of individual fitness and reduced evolutionary adaptability, which may adversely affect the potential of populations to adapt to changes in environmental conditions (Ellstrand and Elam, 1993; Kéry et al., 2000).

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Environmental stochasticity, which is a major threat to small natural populations of plants (Menges, 2000; Matthies et al., 2004), is less likely to be a problem for botanic garden populations, as conditions there are kept close to optimal by gardeners through weeding, watering, fertilizing, the application of pesticides etc. However, such management may lead to genetic changes and adaptation of the ex situ populations to the artificial conditions (Husband and Campbell, 2004). As a consequence, ex situ populations may become maladapted to conditions in the wild, which may adversely affect their suitability as sources for reintroduction efforts. Moreover, gardeners may unconsciously select for certain plant traits and so influence plant evolution.

The problems of genetic erosion and artificial adaptation are well known from captive breeding programmes of animals (reviewed by Frankham (2008) and Williams and Hoffman (2009)). The negative consequences of maladaptations for the success of reintroductions have been studied for various animal taxa, e.g. fish (Heath et al., 2003) and amphibians (Kraaijeveld-Smit et al., 2006). In contrast, little is known about the effects of ex situ conservation of plant species on the genetic diversity of garden populations, potential adaptations to the artificial environment and genetic changes due to unconscious selection.

We investigated the genetic variability and performance of plants from twelve botanic garden and five natural populations of the rare monocarpic perennial *Cynoglossum officinale* L. (Boraginaceae). Genetic variability was analysed using eight nuclear microsatellites. We germinated seeds from all study populations and grew plants in a common garden at two nutrient levels. We recorded fitness-related traits of the vegetative plants in the first year and of the flowering plants in the second year. We addressed the following questions: (1) Is the genetic variation of botanic garden populations lower than that of natural populations, and does it decrease with the duration of cultivation? (2) Has cultivation in botanic gardens resulted in changes of ecologically important traits? (3) Is the performance of plants from botanic gardens lower than that of plants from natural populations, and is it related to genetic variation?

2. Materials and methods

2.1. Study species

We chose *C. officinale* L. (Boraginaceae) as a case study based on the following considerations: *C. officinale* is short-lived and thus more likely to show effects of drift and adaptation to botanic garden conditions than long-lived species, because these effects should increase with the number of generations. The species was available from many botanic gardens, and it is endangered in some German states, but still relatively common in others (Korneck et al., 1996), so that sampling does not threaten the species. Finally, microsatellites had been previously developed for *C. officinale*.

C. officinale is a monocarpic perennial of dry grasslands, ruderal sites, and coastal sand dunes in Europe (de Jong et al., 1990). The plant produces a rosette with a taproot in the first year and if the taproot exceeds a certain threshold size, produces one to several flowering stems in the second year. Flowers open from May to July. *C. officinale* is pollinated by insects, in particular bumblebees, but the plant is self-compatible and geitonogamous selfing is frequent (de Jong et al., 1990; Vrieling et al., 1999).

2.2. Sampling of the populations

Of 26 botanic gardens in Germany and Switzerland contacted in 2007, 12 provided seeds and leaf material for the study. Five natural populations were sampled, Frauenberg (50°45′20N; 8°47′12E) and Amöneburg (50°47′50N; 8°55′12E) in central Hesse near the town of Marburg, and Heidenheim (48°41′17N; 10°08′31E), Giengen (48°38′23N; 10°14′46E) and Nattheim (48°41′42N; 10°11′55E) on the Schwäbische Alb in the south of Germany. The population Heidenheim was small (14 plants), whereas all others consisted of several hundred plants. The minimum distance among populations was 4.3 km. The distance between the two sampling areas is c. 250 km. In every population c. 30 seeds and one leaf per plant were collected from 4–13 individuals, depending on the size of the populations. In the natural populations, along a 40 m transect one plant was sampled every three meters. Leaves were dried in silica gel and stored at room temperature. The botanic gardens were asked to provide information on their populations, in particular concerning the origin of the plants, the number of plants sampled in nature, the duration of cultivation and the minimum population size over the years.

2.3. Microsatellite analysis

In total, 143 plants were screened with eight nuclear microsatellites (C2–19, C2–42, C2–43, C2–62, C2–72, C3–41, C3–79 and the newly defined C2–62_2 (see below)) developed by Korbecka and Wolff (2004). DNA-extraction followed the method of Xin et al. (2003), as modified by Jump and Peñuelas (2006) and the DNA-concentration was determined with a photometer. For PCR, samples were diluted to a concentration of 5 ng/μl. PCRs were carried out using 20 μl reaction volumes. Except for the primer C2–42, each reaction volume contained 5 ng DNA, 2 μl PCR-buffer ($(NH4)SO_4$), 2 mM MgCl, 0.2 mM dNTPs, 2 pmol of each primer (forward primer fluorescence labelled) and 0.5 μ Taq-polymerase. For the primer C2–42 twice the amount of MgCl and Taq-polymerase was used. Reaction volumes were filled to 20 μl with equal parts of water and 10% polyvinylpyrrolidone solution. After 4 min of denaturation at 94 °C, PCR was performed for 35 cycles under the following conditions: 30 s at 94 °C, 45 s at 55 °C and 45 s at 72 °C. A final extension step of 10 min at 72 °C was added. PCR-fragments were separated on 8% acrylamide gels using a Li-COR 4300 DNA Analyser. Size standard was a fluorescence labelled 300 bp ladder (Li-COR, Lincoln, USA). PCR-fragments were analysed using the software package SAGA GT (Li-COR, Lincoln, USA). The primer pair C2–62 produced two independent banding patterns, which were found in all analysed samples. The additional banding pattern, which was not observed in an analysis of *C. officinale* individuals from the costal dunes of the Netherlands with the same markers (G. Korbecka pers. comm.), was treated as an individual duplicated locus (C2–62_2) and included in the analysis.

From the genetic data the mean number of alleles and the percentage of polymorphic loci per population were calculated with GenAlEx 6.0 (Peakall and Smouse, 2006). Allelic richness and Nei's gene diversity were calculated with FSTAT 2.9.3 (Goudet, 1995). The calculation of allelic richness per population was based on a rarefaction sample size of four individuals per population. Linkage disequilibrium between each pair of loci was tested using GENEPOP 4.07 (Rousset, 2008).

2.4. Germination and plant performance

For germination, the pericarp of 18–28 randomly chosen nutlets per mother plant was carefully removed and the seeds put on wet filter paper in Petri dishes and kept at 5 °C for stratification (de Jong et al., 1990). After 33 days, we counted the number of germinated seeds, removed the seed coat from seeds which had not germinated after the stratification treatment, and after a few days in an incubator (20 °C, darkness) again recorded the germination. As soon as they had germinated (from the end of May till beginning