

**THE IMPORTANCE OF RECENT POPULATION HISTORY FOR
UNDERSTANDING GENETIC DIVERSITY IN THREATENED
SPECIES, WITH SPECIAL REFERENCE TO
*DRYOPTERIS CRISTATA***

U. LANDERGOTT^{*1}, G. KOZLOWSKI², J. J. SCHNELLER²
& R. HOLDEREGGER³

¹Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland; ²Botanical Garden of Fribourg, University of Fribourg, Albert-Gockel 3, CH-1700 Fribourg, Switzerland and ³Division of Biodiversity, Swiss Federal Research Institute WSL, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland

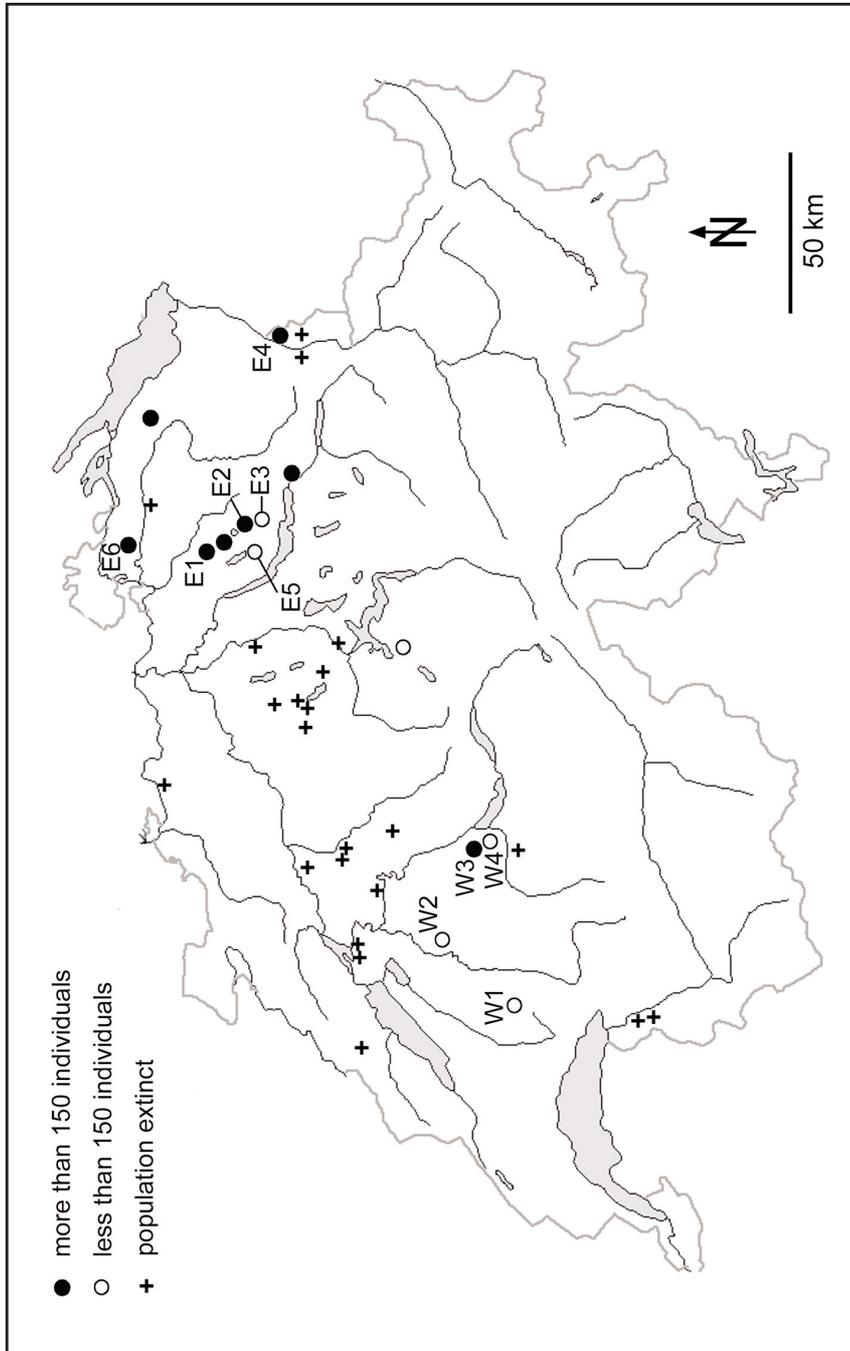
*Correspondence. Urs Landergott, Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland, Tel: +41 1 634 84 36, Fax: +41 1 634 84 03, e-mail: urs.landergott@gmx.net

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ABSTRACT

The maintenance of genetic diversity and stochastic losses of diversity during periods of small population size have become major points of concern in conservation biology. However, empirical research on random evolutionary processes in natural plant populations is still scarce and is reviewed here in comparison to our case study on *Dryopteris cristata*. Detailed recent population histories of this wetland fern have been documented in Switzerland. We found that the lack of correlation between present-day genetic diversity and current population size in this fern, as well as in other newly rare and endangered plant species, is best explained by recent population histories.

Genetic diversity is strongly affected by genetic bottlenecks, which resulted in a loss of about 40% of genetic variation even in the long-lived allotetraploids *D. cristata* and a Hawaiian silversword. In contrast, distinct reductions in population size did not severely reduce genetic diversity in populations of the latter two species in the short-term. Accordingly, there was almost no spatial genetic substructure in populations of *D. cristata*. However, evidence for genetic drift was found in small populations of *D. cristata* and has also been reported for flowering plant species, indicating that small populations are nevertheless prone to random losses of genetic diversity in the long-term. This short review elucidates the importance of recent population history for both population genetics and conservation biology. Understanding population history can substantially improve predictions on the genetic diversity in remnant populations of threatened species. Further studies on natural populations of plant species with different life cycles and ploidy levels remain valuable.



Map 1. Present and former distribution of *Dryopteris cristata* in Liechtenstein and Switzerland (Landergott *et al.*, 2000), with location of the ten populations included in the analysis of genetic diversity (population abbreviations see Table 1).

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TABLE 1. Location and abbreviation of the ten genetically studied Swiss populations of *Dryopteris cristata* with historical population sizes and census number in 1999, category of population history, molecular variance and Mantel test for the correlation (r) and its significance (P) between spatial distances and genetic distances among 20 individuals per population.

Population abbreviation	Location	Population size ¹				Population history ²	Molecular variance	Mantel test	
		1881-1945	1946-1979	1980-1998	1999			r	P
W1	Sâles	S	BN (2)	s	60	I	0.134	0.017	0.400
W2	Düdingen	L	s	S	60	II	0.534	0.081	0.081
W3	Schmittmoos	—	—	—	>500		0.355	0.013	0.431
W4	Reutigen	L	s	s	110	II	0.734	0.021	0.341
E1	Wildert		S	I	>500	III	0.311	0.108	0.128
E2	Robenhuserriet	S	L	L	>500	III	0.384	-0.083	0.260
E3	Ambitzgi	BN (20)	s	s	130	I	0.197	0.326	0.007
E4	Ruggellerriet	L	L	L	300-500		0.311	0.099	0.125
E5	Greifensee			BN (3)	60	I	0.134	-0.061	0.343
E6	Barchetsee			BN	250	I	0.218	-0.139	0.121

¹Population size classes according to Landergott *et al.* (2000): BN = bottleneck of less than 25 individuals (exact records are given in parentheses if available); S = small population with less than 150 individuals; L = large population with more than 150 individuals; lower case letters represent estimates, which were deduced from general remarks on habitat conditions without relying on direct records of *D. cristata*.

²Types of recent population history: I = with a historical bottleneck; II = reduction of a formerly large population to a small one before 1945; III = increase of a formerly small population to a currently large one.

INTRODUCTION

Genetic differentiation can be the result of local adaptation, genetic variation may be correlated with fitness, and genetic diversity is a prerequisite for future selection and adaptation (Booy *et al.*, 2000). The maintenance of genetic diversity has consequently become a central focus of concern in conservation biology (Ellstrand & Elam, 1993). Resources for conservation efforts are limited, and accurate predictions regarding the genetic diversity in populations of threatened species are needed. In this context, the stochastic loss of genetic diversity during periods of small population size is an important factor (Barrett & Kohn, 1991). Genetic diversity has been found to be positively correlated with present-day population size in some plant species (e.g. Ellstrand & Elam, 1993; Raijmann *et al.*, 1994; Fischer & Matthies, 1998). However, no such correlation has been reported for several rare and endangered species, and this outcome has often been suggested to be due to assumed changes of population size in recent population history (Ellstrand & Elam, 1993; Kull & Paaver, 1997; Kahmen & Poschlod, 2000; Lutz *et al.*, 2000; Schmidt & Jensen, 2000; Fréville *et al.*, 2001; Podolsky, 2001). As recent historical population sizes usually remain unknown, empirical research on the effects of genetic bottlenecks and drift on wild plant populations is almost lacking (Booy *et al.*, 2000; but see Richards *et al.* 2003).

The stochastic loss of genetic diversity (specifically, allelic richness) associated with a bottleneck has been studied in artificially founded populations of *Sarracenia purpurea* (Schwaegerle & Schaal, 1979), the Hawaiian Mauna Kea silversword, *Argyroxiphium sandwicense* ssp. *sandwicense* (Friar *et al.*, 2000) and *Rutidosia leptorrhynchoides* (Young & Murray, 2000). A distinct founder effect has also been reported for a population of *Cypripedium calceolus*, which existed for presumably no more than 200 years (Kull & Paaver, 1997), and for a single-founder population of *Trifolium amoenum* (Knapp & Connors, 1999). Accordingly, out of 13 studied populations of the locally rare *Pedicularis palustris*, lowest genetic variation has been reported for a nowadays large population of recent origin (Schmidt & Jensen, 2000). By comparing cytoplasmic diversity in a population of *Thymus vulgaris* before and after fire, Manicacci *et al.* (1996) showed that disturbances may severely reduce genetic diversity. Founder effects may locally cause a shift from nucleo-cytoplasmic to purely cytoplasmic determination of sex and lead to high frequencies of females in colonising populations of the gynodioecious *T. vulgaris* (Manicacci *et al.*, 1996). The stochastic nature of founding events has also been documented in *Silene latifolia* with younger populations displaying higher genetic differentiation than older ones (McCauley *et al.* 1995). In common species with substantial gene flow among populations, however, genetic diversity can be restored rapidly with time after a founder event (von Flüe *et al.*, 1999; Richards *et al.* 2003).

In ferns, the relation between population history and genetic diversity has been discussed with respect to the colonisation of patchy rock habitats (Holderegger & Schneller, 1994; Schneller & Holderegger, 1996a; Vogel *et al.*, 1999). Genetic diversity has been found to be positively correlated with population age in *Asplenium ruta-muraria*, indicating initial single spore colonisation and subsequent multiple colonisation events with increasing population age (Schneller & Holderegger, 1996a).

Another opportunity for studying effects of recent population history on present-day genetic diversity is offered by the population dynamics of threatened species caused by man-made habitat disturbances. For the locally rare and endangered wetland fern *Dryopteris cristata*, recent population histories in Switzerland could be reconstructed

(Lander Gott *et al.*, 2000) and present-day genetic diversity assessed (Lander Gott *et al.*, 2001). Here, additional data are presented on the spatial genetic structure within populations of *D. cristata*. Our aim is to evaluate, illustrate and discuss the results of this case study from the perspective of conservation biology. We discuss implications for conservation in general by including comparable, but scarce, empirical studies on the influence of recent population history on present-day genetic diversity in newly rare and endangered plant species.

DRYOPTERIS CRISTATA-SPECIFIC BACKGROUND

The Crested Buckler fern, *D. cristata* (L.) A. Gray, has become rare and endangered in southwestern Central Europe (references in Lander Gott *et al.*, 2000). In Switzerland, at the southern border of the species' European distribution, 22 (62% of all described) populations are extinct due to habitat destruction, and only 14 populations remained in 1999 (Map 1; Lander Gott *et al.*, 2000). The habitats of the surviving populations are best characterised as different remnants of formerly exploited, but not totally destroyed peat bogs. The commercial exploitation of peat bogs until approximately 1945 and their subsequent management as conservation areas caused substantial changes in population sizes of *D. cristata*. In a previous study, we reconstructed fluctuations in most of the Swiss populations of *D. cristata* over 120 years using herbaria and literature data (Lander Gott *et al.*, 2000). However, even for this attractive fern species, and in a study area with a rich floristic tradition, historical records of population sizes remained incomplete (Table 1). By including current population sizes determined in a field survey in 1999, it was nevertheless possible to establish three types of recent population histories: (I) the occurrence of a severe historical bottleneck of less than 25 individuals, (II) the reduction of a formerly large population (more than 300 individuals) to a small remnant (less than 150 individuals) before 1945 and (III) the increase of a formerly small population to a presently large one (Table 1; Lander Gott *et al.*, 2000).

We estimated genetic diversity in 14 populations of *D. cristata* from Switzerland and southern Germany by random amplified polymorphic DNA (RAPDs; Lander Gott *et al.*, 2001). In each of the 14 studied populations, 20 individuals were randomly sampled throughout the population area. This sample size is recognised as sufficient to provide accurate genetic diversity estimates for plant populations (Nybom & Bartish, 2000). RAPD diversity of *D. cristata* was extraordinarily low in the study area (for discussion see Lander Gott *et al.*, 2001). However, the detected genetic variation within populations was not correlated with current population sizes (Figure 1; Table 1). In a hierarchical analysis of molecular variance (AMOVA), 15% of total variance was attributed to variation among three geographic regions (western and eastern Switzerland and southern Germany), 34% to variation among populations within regions, and only 51% to variation within populations. High population differentiation was indicated by a F_{ST} -value of 0.49 as well, and genetic divergence among populations was not correlated with geographic distances. These findings suggested very limited gene flow among populations of *D. cristata*. The absence of gene flow as an equalising force and the assumed selective neutrality of RAPD markers allowed us to investigate the effects of random evolutionary processes in recent population history on the genetic diversity in natural populations of *D. cristata*. Note that the categorisation of population size was deduced from the distribution of census numbers found in the studied populations (Lander Gott *et al.* 2000). Population size classes (Table 1) are thus somewhat arbitrary, and critical population sizes are likely to be different for other plant species with

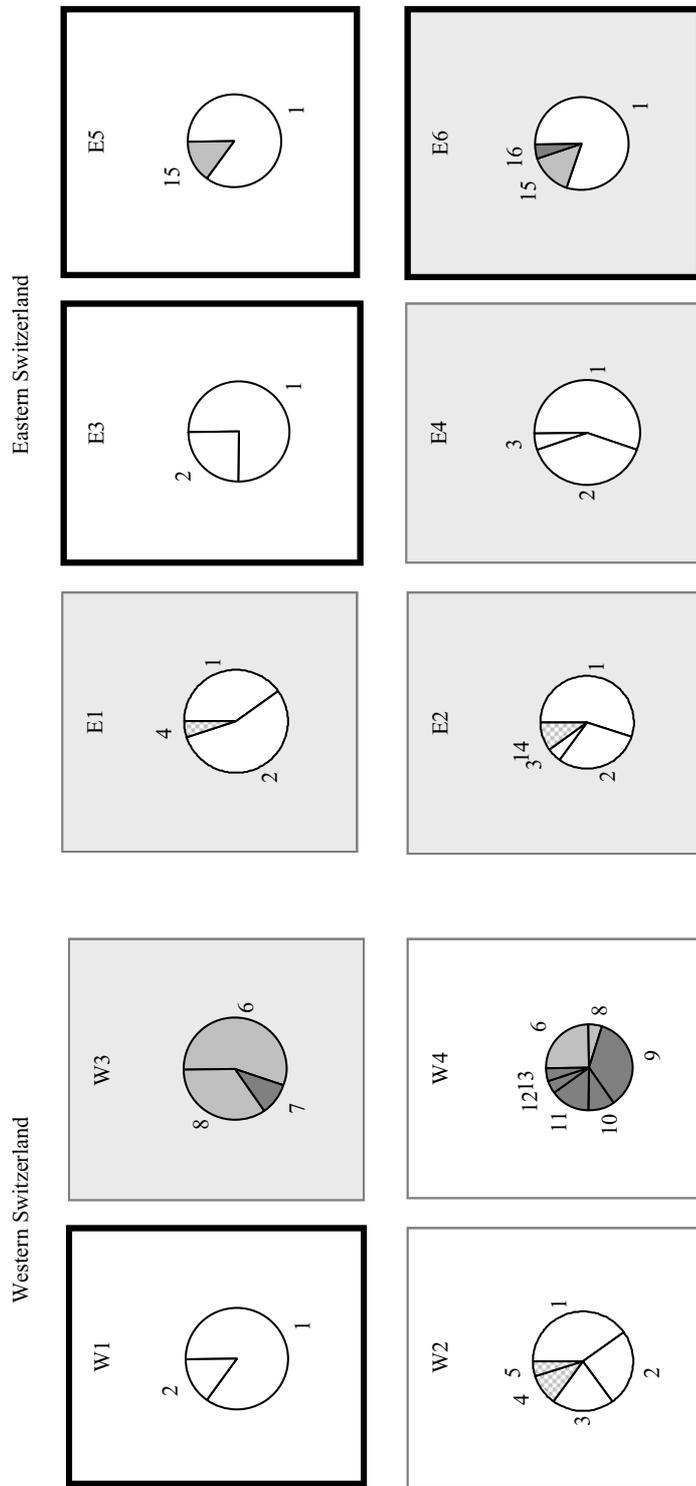


Figure 1. Frequencies and distribution of 16 RAPD multi-band phenotypes in the ten studied populations of *Dryopteris cristata* from western and eastern Switzerland according to Landergott et al. (2001). RAPD phenotypes No. 1, 2, 3, 5 and 14 were found insouthern Germany as well (data not shown). White phenotypes occurred in all three geographical regions, hatched phenotypes occurred in two regions, faint grey phenotypes were restricted to single regions and dark grey phenotypes wererestricted tosingle populations. Large populations with more than 150 individuals are underlined with a grey background,and populations with recent historical bottlenecks of less than 25 individuals are marked with bold border lines (population abbreviations see Table 1).

different life histories and ploidy levels. As no information was available on historical population sizes of *D. cristata* from southern Germany (Lander Gott *et al.*, 2001), the following considerations will focus on the ten Swiss populations included in the RAPD analysis (Map 1; Table 1).

THE PAST EXPLAINS THE PRESENT

In conservation biology, the number of multi-locus genotypes present in a population has been supposed to be a more important measurement of genetic diversity than the number of single variable markers (Schneller & Holderegger, 1996b). Molecular variance (Table 1) is based on genetic distances among RAPD multi-band phenotypes and on their numbers and frequencies (Excoffier *et al.*, 1992; Fischer & Matthies, 1998), which are visualised for the ten study populations of *D. cristata* in Figure 1.

Present-day RAPD diversity in populations of *D. cristata* was most prominently affected by recent historical genetic bottlenecks (population history type I; Table 1). In populations E3 and W1, severe bottlenecks were caused by peat exploitation. Only 20 individuals survived in population E3 in 1892, at the same place where we found 130 individuals in 1999 (Table 1; Lander Gott *et al.*, 2000). At Sâles (population W1), *D. cristata* was considered to be abundant in 1905, but only a small population was reported in 1929, which then has been argued to become extinct due to further habitat destruction in the near future. By 1959, only two remnant individuals were reported from there, but in 1999 we found again 60 individuals at Sâles (Lander Gott *et al.*, 2000). In contrast, genetic bottlenecks in populations E5 and E6 were most probably due to the recent establishment of new populations by a few founder individuals (Table 1). This could be inferred from the fact that both localities were thoroughly investigated before *D. cristata* had been reported there for the first time (Lander Gott *et al.*, 2000). All four populations with recent bottlenecks were characterised by significantly decreased genetic variation, as compared to populations without bottlenecks (Table 1; *U*-test, $P < 0.02$; Lander Gott *et al.*, 2001). Furthermore, the dominance of a widespread RAPD multi-band phenotype was a common feature of these recently bottlenecked populations of *D. cristata* (frequency of phenotype No. 1 ≈ 0.75 in all four cases; Figure 1). They comprised less rare RAPD phenotypes than most of the other studied populations (Lander Gott *et al.*, 2001). The stochastic loss of genetic diversity during severe bottlenecks has been predicted by population genetic theory for diploid, outbreeding organisms (e.g. Barrett & Kohn, 1991). For polyploid species, however, less of an effect of a bottleneck on the genetic diversity would be expected, because high levels of segregational heterozygosity could nevertheless be maintained within populations (Barrett & Kohn, 1991; Bretagnolle *et al.*, 1998). In contrast, the four recently bottlenecked populations of the allotetraploid fern *D. cristata* ($2n = 164$) showed a substantial loss of genetic variation (reduction in molecular variance = 40%; Table 1) as compared to populations without bottlenecks. A similar loss of genetic diversity, as measured by the number of microsatellite alleles within population (reduction = 36%) and the proportion of polymorphic loci (reduction = 43%) has been reported in the allotetraploid Mauna Kea silversword *A. sandwicense* ssp. *sandwicense* after a genetic bottleneck of two individuals, which was associated with the populations re-introduction (Friar *et al.*, 2000).

In *D. cristata* from Switzerland, highest genetic variation was found in two currently small populations W2 and W4 (molecular variances = 0.534 and 0.734, respectively; Figure 1; Table 1). These populations were large ones at the beginning of

the 20th Century, but subsequently suffered a severe reduction in population size due to peat exploitation, leaving as few as 50 to 150 individuals of *D. cristata* in small marginal habitats by 1945 (population history type II; Table 1; Landergott *et al.*, 2000). Similar genetic variation was detected in a putatively old large population from southern Germany (B4, molecular variance = 0.655; Landergott *et al.*, 2001). Unfortunately, there were no populations documented to have always been large during the past 120 years, except for the geographically isolated population E4 (Landergott *et al.*, 2001). However, the comparatively high molecular variance still maintained in the small populations W2 and W4 of *D. cristata* suggested that a distinct reduction in population size to less than 150 individuals did not substantially reduce genetic variation in populations of this long-lived, homosporous fern species in the short term. Similarly, a reduction in size to fewer than 50 plants in the remnant natural population of the long-lived Mauna Kea silversword was not accompanied by a significant loss of genetic diversity (Friar *et al.*, 2000). High genetic variation was also maintained in large and small remnants of formerly large metapopulation systems of *Cypripedium calceolus* (Kull & Paaver, 1997) and *Pedicularis palustris* (Schmidt & Jensen, 2000) and in fragmented subpopulations of *Haplostachys haplostachya* (Morden & Loeffler, 1999). In *Clarkia dudleyana*, a historically large but currently small population exhibited still high genetic variation, and classifying this population as either a small or large one has been reported to alter several trends of correlations between population size and various measures of genetic diversity (Podolsky, 2001).

A prerequisite for the maintenance of a considerable amount of genetic diversity in small population-remains is the random distribution of genotypes within populations. For several predominantly outcrossing seed plants, weak spatial genetic structuring within populations has been reported (Heywood, 1991). Even less spatial genetic substructure would be expected in populations of long-lived, homosporous ferns, due to their high spore production and great potential for long-distance spore dispersal (Cousens, 1988). To get some insights into the spatial genetic structure within populations of *D. cristata*, we performed Mantel tests based on the spatial distances and the squared Euclidean genetic distances among the 20 RAPD phenotypes for each of the 14 studied populations separately (with 999 permutations using NTSYS-pc; Rohlf, 1998). A significant positive correlation was found only in the recently bottlenecked population E3 (Table 1; data of four German populations not shown). Because of the small sample size per population and because Mantel tests do not have high resolving power to detect spatial genetic structure within populations (Heywood, 1991), results should be interpreted with caution. However, at a larger spatial scale, they corroborate the proposed maintenance of considerable genetic variation in small remnants of formerly large populations of *D. cristata*. Furthermore, in small populations where a larger proportion of individuals has been sampled, they indicate little or no spatial genetic structure at the small scale. In contrast, positive autocorrelations have been observed at the small spatial scale in some populations of the rock fern species *Pteris multifida* (Murakami *et al.*, 1997) and *Asplenium trichomanes* ssp. *quadrialeans* (Suter *et al.*, 2000). Further studies of spatial genetic structure and its relation to breeding systems in natural populations are needed for general predictions on the maintenance of genetic variation in small population-remains of ferns, but also in flowering plants (Stehlik & Holderegger, 2000).

Despite the comparably high level of genetic variation maintained in some small populations of *D. cristata*, these populations could be prone to future stochastic losses

of genetic variation through random genetic drift (Barrett & Kohn, 1991). A theoretical prediction on genetic drift states that allele frequencies within populations fluctuate and tend to drift apart, while overall average allele frequencies among populations remain constant (Hartl & Clark, 1997). In currently small and/or recently bottlenecked populations, deviations of RAPD marker frequencies within populations from their overall frequencies were in fact significantly increased, compared with marker frequency deviations in large populations (Lander Gott *et al.*, 2001). This gave evidence that small populations of *D. cristata* are actually under genetic drift, which might lead to random loss of alleles in the future. Severe genetic erosion due to drift occurred in the short term in small populations of the short-lived *S. latifolia* when significant gene flow was absent (Richards *et al.* 2003). Morden and Loeffler (1999) reported a substantial increase in the number of RAPD markers either present or absent in all individuals of the smallest subpopulation of *Haplostachys haplostachya* relative to other subpopulations, suggesting that drift was moving this subpopulation towards fixation of alleles. Kull and Paaver (1997) emphasised remarkable fluctuations of allele frequencies among isolated remnant populations of *Cypripedium calceolus* as well. Further evidence for genetic drift in small natural populations has been observed in *Salvia pratensis* and *Scabiosa columbaria* as judged from substantially higher genetic differentiation among small populations than among large ones (van Treuren *et al.*, 1991). Accordingly, greater genetic differentiation among populations of *Cyclamen balearicum* from habitat islands in southern France than among populations from the true Balearic islands (Affre *et al.*, 1997) has been attributed to genetic drift in small relict populations following habitat fragmentation in southern France in the past 500 years (Thompson, 1999).

Finally, the intermediate level of genetic variation found in two of the largest populations of *D. cristata*, E1 and E2, could also be explained by their shared population history (type III; Table 1). These populations had first been recorded as small ones and substantially increased in size in the second half of the 20th Century (Lander Gott *et al.*, 2000), presently showing high viability as indicated by spore production (Lander Gott, personal observation). Hence, ecological factors might be more important for short-term population viability in *D. cristata* than genetic diversity as assessed by neutral markers. Accordingly, viable populations with complete allozyme uniformity have been reported for the inbred, polyploid ferns *Asplenium ruta-muraria*, *A. septentrionale* and *Polypodium vulgare* (Schneller & Holderegger, 1996b). Notice that the relevance of neutral genetic variation for conservation purposes is presently under discussion (Crandall *et al.*, 2000; Fischer *et al.*, 2000; Podolsky, 2001), but in the end, adaptively significant genetic variation will be affected by stochastic losses during periods of small population size in much the same way as neutral variation.

CONCLUSIONS

As far as *D. cristata* is concerned, its extraordinarily low overall genetic diversity, somehow conflicting with its high genetic population differentiation, hinders straightforward conclusions for the species' conservation. However, this fern is presently stated to be vulnerable (IUCN, 1994) in the region of western Switzerland (Lander Gott *et al.*, 2000) and it is the focus species of a local conservation project at the Botanical Garden of Fribourg in Switzerland (Kozłowski, 1999). Without knowledge on possible adaptive differences among populations, the best strategy maintains genetically distinct populations. For example, in western Switzerland, the RAPD

phenotypes of population W1 are a subset of W2 and, likewise, those of W3 are essentially a subset of W4 (Map 1; Figure 1). Preservation of genetic diversity in this region requires priority conservation of populations W2 and W4 (Figure 1; Table 1). Their small sizes make them prone to genetic drift, however, and *in situ* increases of their population sizes are thus desirable. Unfortunately, the autecology of *D. cristata* is poorly known (Page, 1997). Studies on its breeding system, safe sites, and recruitment of individuals in natural populations would considerably improve conservation strategies for this threatened fern species.

In general, the studies reviewed in this article demonstrate the importance of recent population history for both population genetics and conservation biology. Especially in newly rare, threatened species, substantial recent historical changes in population size should be expected. Therefore, conservation practice requires an understanding of the effects of stochastic forces on genetic diversity (Barrett & Kohn, 1991), and theoretical predictions on random evolutionary processes during periods of small population size need to be tested in natural populations of plant species with different life histories and ploidy levels. Since population history is often incompletely known, the empirical base is still small and further case studies that consider genetic diversity in the light of population history will be most worthwhile. Floristic records in herbaria and in the literature, as well as continuous monitoring of small and large natural populations are important in this regard. If adequate records are available, population history can substantially improve predictions on genetic diversity in remnant populations of threatened species and thereby help in choosing priority populations for conservation.

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