

Genetic diversity of *Carex davalliana* and *Succisa pratensis* in mown and abandoned fen meadows

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Summary

1 We studied the effects of secondary succession on the genetic diversity of two common fen plant species. Starch gel electrophoresis was used to assess allozyme diversity in *Carex davalliana* and *Succisa pratensis*. Diversity was then compared among intact, mown fen meadows and a chronosequence of abandoned fen meadows.

2 Genetic variation at the population level was similar to the expected values for these taxa irrespective of management regime. Cessation of mowing had no effect on genetic diversity of both species: none of the measured variables (percentage of polymorphic loci, alleles per locus, alleles per polymorphic loci, observed and expected heterozygosity) differed significantly between mown and abandoned meadows. Moreover, time since abandonment (up to 35 years) had no influence on genetic variation.

3 These results indicate that observed changes in growth and reproductive traits in the field are phenotypic and do not reflect selection for or loss of genotypes. Since 35 years of abandonment did not erode the genetic diversity of these species, there seems to be a high probability to restore the former state of a fen meadow by re-establishment of traditional management.

Keywords: abandonment, allozyme variation, calcareous fens, secondary succession

Bulletin of the Geobotanical Institute ETH (2002), **68**, 45–54

Introduction

Since 1850, roughly 90% of all Swiss wetlands have disappeared (BUWAL 1990). The remaining ones are threatened by land-use changes, such as intensified farming, habitat fragmentation or abandonment. Wetlands and especially calcareous fen meadows contain a high species richness and harbour many rare plant species (Wheeler 1988; Landolt 1991). Nearly 50% of the threatened

plant species of Switzerland occur in fen meadows (BUWAL 1990). To be able to retain this species richness, fen meadows depend on continued, but low-intensity agricultural use such as late-season mowing.

Cessation of mowing promotes secondary succession, leading to the replacement of characteristic by ubiquitous species and the invasion of tall herbs, shrubs and trees. Sev-

eral studies have assessed the demographic and morphological changes following the cessation of mowing. While short-term abandonment can promote dominant graminoids and late blooming forbs (Diemer & Pfadenhauer 1987; Bosshard *et al.* 1988; Schopp-Guth *et al.* 1994) long-term abandonment clearly leads to a decline in species richness (Preiss 1982; Diemer *et al.* 2001). Especially the build-up of litter, which reduces canopy and soil temperatures, leads to a decline of prostrate rosette species such as *Parnassia palustris* (Bosshard *et al.* 1988). Moreover, it has an inhibitory effect on germination and establishment of seedlings (Maas 1988; Bosshard *et al.* 1988; Jensen 1997), which leads to a further decrease of populations. If populations become small, it is likely that genetic diversity decreases as well. However, as far as we know, no study ever examined the genetic changes in established populations after abandonment by analysing genetic diversity directly. Gray *et al.* (1985) assessed genetic diversity of *Agrostis curtisii* invading heathland during post-fire succession and found no clear patterns. Hartnett & Bazzaz (1985) showed that the genetic composition of populations of *Solidago canadensis* remains stable in successional environments. On the other hand, Bazzaz (1996) concludes from several morphological studies that genetic variation within populations probably declines during old-field succession. However, this conclusion is based on studies of invading species and may not hold for established populations of habitat specialists. Billeter (2001) found a clear effect of abandonment on seedling density and on the morphology of plants from established populations in fens, but all observed effects vanished when plants were grown in a common garden. Billeter (2001) therefore suggests that the demographic and morphological changes observed

in the field are mainly phenotypic responses to litter build-up and canopy structure. The main goal of this study is to directly assess the impact of abandonment on genetic diversity within established populations to verify the absence of genetic change postulated by Billeter (2001).

We chose two abundant fen indicator species for this study rather than rare species. Although rare species may be more responsive to abandonment, we believe that community maintenance depends on the vitality and persistence of abundant species (akin to the keystone species concept as defined by Paine 1966). If abundant species suffer from abandonment, it is likely that rare species are threatened too. The two species chosen for this study, *Carex davalliana* and *Succisa pratensis*, are both abundant in montane fen meadows; they differ in life history and morphology so that they can be regarded as representative of a wider range of fen meadow species.

The main questions for this study are: What is the level of genetic diversity in the two plant species? Does genetic diversity decrease after abandonment? Can we identify temporal effects related to time since abandonment?

Materials and Methods

FIELD SITES

The focus of this study are fen meadows of the phytosociological alliance *Caricion davallianae* (Ellenberg 1996). To assess the impact of abandonment on *Succisa pratensis* and *Carex davalliana* we selected 16 fallow fen meadows and 6 mown fen meadows from an inventory containing all protected fens of Switzerland (BUWAL 1990). We restricted our research area to the northeastern part of Switzerland and to montane fens (altitude > 800 m a.s.l.,

Table 1. Designation and relevant characteristics of the study sites (montane fens in northeastern Switzerland): age of fallows (years since abandonment, age 0 = mown fens), altitude in m a.s.l., slope in degrees, aspect, and soil pH (determined in deionised water).

Canton	Site	age (years)	altitude (m a.s.l.)	slope (°)	aspect	soil pH
AR	Foren I	12	1030	11.3	N	5.8
AR	Foren II	10	1040	11.3	N	5.4
AR	Foren III	33	1050	2.5	N	5.8
AR	Foren IV	0	970	2.5	N	5.7
SG	Altschenchopf I	25	1280	20.0	E	7.4
SG	Altschenchopf II	0	1300	17.5	E	6.6
SG	Rossweid	20	1100	16.3	N	6.8
SG	Salomonstempel	20	1020	13.8	N	6.8
SG	Chellen	0	1100	5.0	S	5.7
SZ	Bergliboden	9	1050	11.3	W	6.6
SZ	Hirzegg	35	1250	12.5	N	6.4
SZ	Mutzenwald	0	960	15.0	NW	5.5
SZ	Bueffen I	10	1240	13.8	N	7.1
SZ	Bueffen II	4	1200	16.3	N	6.4
SZ	Chappelried I	7	1260	18.8	E	5.9
SZ	Chappelried II	30	1240	17.5	NW	6.0
SZ	Chappelried III	35	1280	16.3	E	6.0
SZ	Chappelried IV	0	1250	15.6	E	6.3
SZ	Langried	30	1280	17.5	W	5.5
SZ	Rund Blät I	25	1270	15.0	W	5.8
SZ	Seiler/Zwäcken	15	1320	18.8	NW	6.7
SZ	Rund Blät II	0	1200	20.0	W	6.1

Fig. 1). Fen meadows were selected from seven different regions in the cantons St. Gallen, Schwyz and Appenzell. Within regions one to three abandoned sites were matched with one nearby intact, annually mown fen meadow, which served as control. Fallows varied in age since abandonment (between 4 and 35 years), so that a possible time-dependence of the effects of abandonment could be detected. For a detailed description of the sites, see Table 1.

In each fen meadow, we randomly placed four permanent plots (2 m x 1 m) in May or

June 1998. To assure that the two studied plant species were present in all plots, we randomly replaced plots if either either species was absent.

STUDY SPECIES

Carex davalliana SM. (Cyperaceae) is a characteristic clonal graminoid in montane fens of NE Switzerland, where it often dominates the vegetation. It is a dioecious species, although hermaphrodite tussocks are occasionally found. Plants flower in April and produce on average 15 seeds per female flowerhead

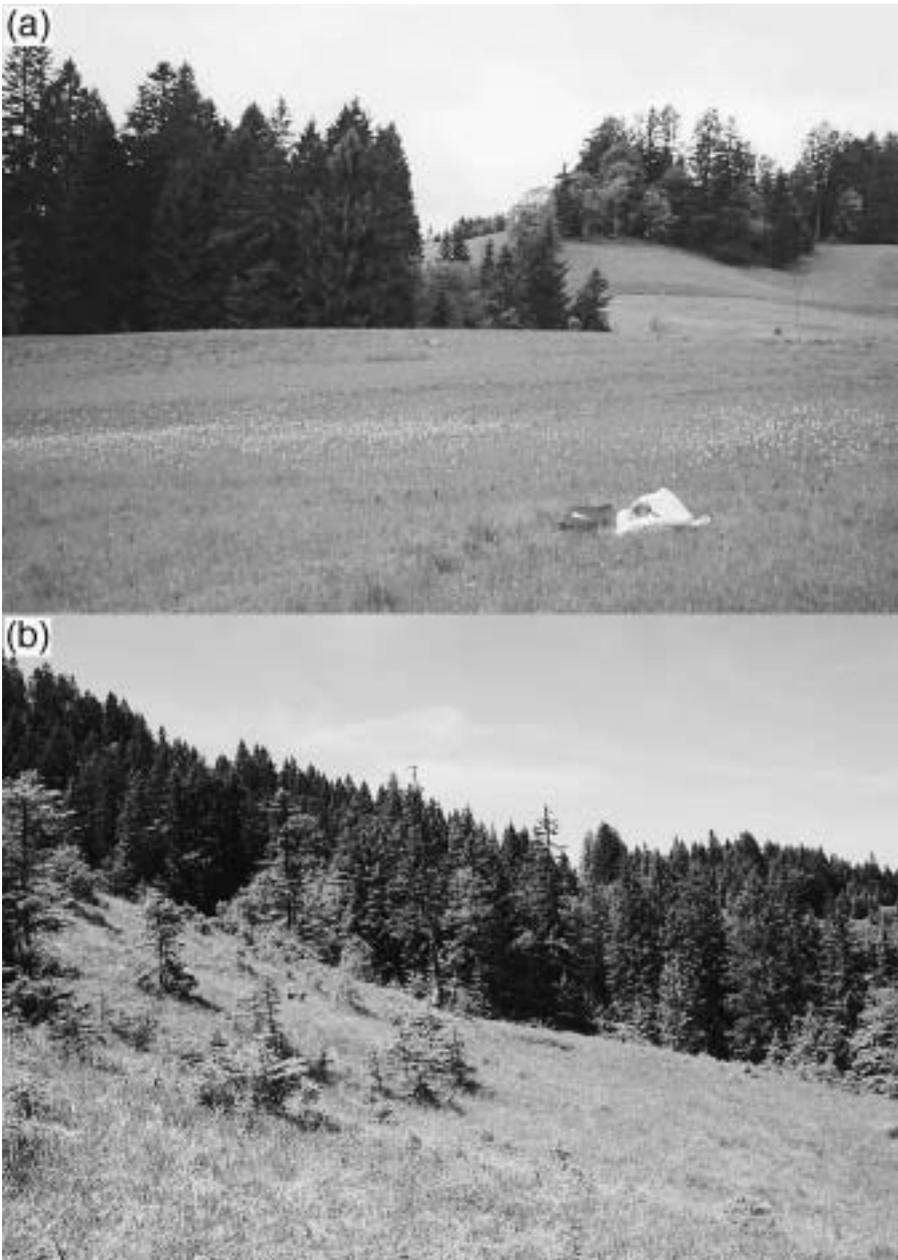


Fig. 1. Typical montane fens in Switzerland: (a) 'Chellen', an annually mown, species-rich fen; and (b) 'Hirzegg', a fen where mowing was abandoned 35 years ago, with accumulation of litter, reduced species richness and spontaneous reforestation.

Table 2. Buffer systems used for starch gel electrophoresis and enzymes analysed in each of the two species. See Appendix 1 for full names and E.C. numbers of enzymes.

Buffer system	Enzymes	
	<i>Carex davalliana</i>	<i>Succisa pratensis</i>
LiOH-borate pH 8.3	AAT-1, AAT-2, AAT-3, ADH, GPI, IDH-1, IDH-2, TPI-1, TPI-2	GPI-1, GPI-2, ME, PGM-1, PGM-2, TPI-1, TPI-2
Morpholine-citric acid	ACO, PGD, ME, PGM, G6PD, MDH	ADH, FBP, IDH, MDH, PGD

(Billeter 2001). Tussocks of this species can reach diameters of up to 30 cm and produce several hundred tillers (Billeter 2001).

Succisa pratensis Moench (Dipsacaceae) is one of the most common rosette-forb species in these fens. It is a long-lived, non-clonal, monoecious species, which flowers in August/September. The rosette produces one to four shoots that can grow up to 1 m in height (Billeter 2001). One shoot forms on average one to three inflorescences (Billeter 2001). The flowers are pollinated by insects and seeds ripen in September/October.

POPULATION SAMPLING AND ELECTROPHORETIC PROCEDURES

Leaf material from 12 plants per site (three randomly chosen plants per permanent plot) was collected for genetic analyses. In addition, we randomly sampled another 18 plants outside permanent plots. For *C. davalliana*, we collected samples from 20 sites, i.e. 600 samples in total. As two sites did not contain *S. pratensis*, only 18 populations (i.e. 540 individual plants) were sampled for this species.

The leaf material was stored on ice during collection and transferred to the refrigerator as soon as possible. All samples were ground in extraction buffer within three days after collection. We used Tris-HCL grinding buf-

fer-PVP solution (Soltis *et al.* 1983), adding Dimethylsulfoxide (DMSO) for extraction, and absorbed the extracts onto filter paper wicks. Wicks were stored in Eppendorf tubes at -80°C and transferred directly from the freezer to starch gels for electrophoresis.

The starch gel (12% w/v) was prepared according to Wendel & Weeden (1989) with minor modifications. We performed horizontal starch gel electrophoresis according to Soltis *et al.* (1983) and Wendel & Weeden (1989), using the two buffer systems LiOH-borate pH 8.3 (Soltis *et al.* 1983), and Morpholine-citric acid (Werth 1991). Recipes used for enzymes were prepared according to Soltis *et al.* (1983) with minor modifications, except for AAT, which was prepared according to Wendel & Weeden (1989). We could resolve 15 loci from eleven enzymes for *C. davalliana* and 12 loci from nine enzyme systems for *S. pratensis* (Table 2).

STATISTICAL ANALYSIS

To calculate statistics of genetic diversity, we used the computer program GDA (Lewis & Zaykin 2000). We estimated the following parameters at the population level: proportion of polymorphic loci (P), mean number of alleles per locus (A), mean number of alleles per polymorphic locus (A_p), mean number of

genotypes per locus (N_g), observed heterozygosity (H_o) and expected heterozygosity (H_e).

We applied a hierarchical linear model to test the effects of region, management status (fallow vs. control) and age of fallow on genetic diversity. Region and management status were nominal variables, while age was a continuous variable nested within management status (see Billeter & Diemer 2000). If necessary, variables were transformed to obtain a normal distribution of residuals. All analyses were done using Genstat 5.0 (Payne *et al.* 1995).

Results

Genetic diversity varied considerably among populations of *Carex davalliana* and *Succisa*

Table 3. Summary of allozyme variation in populations of *C. davalliana* and *S. pratensis* from fens abandoned for 4–35 years and from mown fens (means \pm SE), with the significance (*P*-values) of differences. Abbreviations are: *P*, proportion of polymorphic loci, *A*, mean number of alleles per locus, *A_p*, mean number of alleles per polymorphic locus, *N_g*, mean number of genotypes per locus, *H_o*, observed heterozygosity; and *H_e*, expected heterozygosity.

	Abandoned fens	Mown fens	<i>P</i>
<i>Carex davalliana</i>			
<i>P</i>	0.46 \pm 0.03	0.51 \pm 0.03	0.32
<i>A</i>	1.52 \pm 0.04	1.61 \pm 0.05	0.27
<i>A_p</i>	2.13 \pm 0.04	2.19 \pm 0.07	0.48
<i>N_g</i>	1.81 \pm 0.04	1.88 \pm 0.08	0.41
<i>H_o</i>	0.108 \pm 0.01	0.133 \pm 0.02	0.17
<i>H_e</i>	0.137 \pm 0.01	0.153 \pm 0.01	0.37
<i>Succisa pratensis</i>			
<i>P</i>	0.51 \pm 0.02	0.52 \pm 0.05	0.54
<i>A</i>	1.73 \pm 0.05	1.84 \pm 0.08	0.17
<i>A_p</i>	2.44 \pm 0.07	2.62 \pm 0.09	0.13
<i>N_g</i>	1.91 \pm 0.08	2.03 \pm 0.15	0.33
<i>H_o</i>	0.187 \pm 0.09	0.204 \pm 0.02	0.40
<i>H_e</i>	0.180 \pm 0.01	0.183 \pm 0.02	0.83

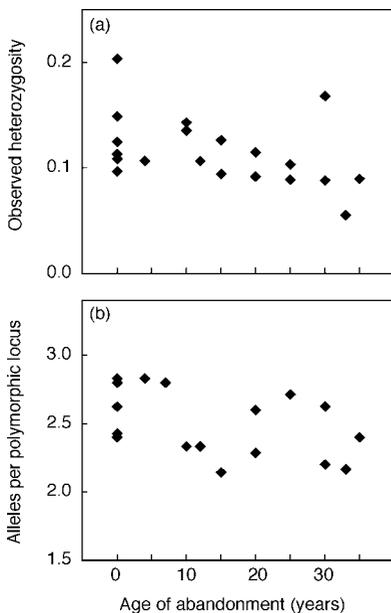


Fig. 2. Genetic diversity, measured as (a) observed heterozygosity (H_o) in populations of *C. davalliana* and (b) mean number of alleles per polymorphic locus (*A_p*) in populations of *S. pratensis*, in relation to the age of fallows (years since abandonment, age 0 = mown fens). Each dot represents one population.

pratensis. In both species, all measures of genetic diversity tended to be lower in populations from fallows than in those from mown fens (Table 3). However, differences were not statistically significant for any of the variables (Table 3). In addition, none of the traits was significantly related to the age of abandonment (all $P > 0.44$). This is illustrated in Fig. 2 for the variables which had differences between fallows and controls closest to significance, i.e. observed heterozygosity in *C. davalliana* (Fig. 2a) and the mean number of alleles per polymorphic locus in *S. pratensis* (Fig. 2b). None of the measures of genetic variation differed significantly among the regions ($P > 0.45$ for *C.*

davalliana, $P > 0.11$ for *Succisa pratensis*, data not shown).

Discussion

Carex davalliana is a monocotyledonous, wind pollinated species, which has levels of within-population genetic variation ($P = 47.5\%$, $H_e = 0.142$) similar to the average values that Hamrick & Godt (1989) calculated for monocot taxa ($P = 40.3\%$, $H_e = 0.144$). The high percentage of polymorphic loci (47.5%) suggests that outcrossing is quite common in *C. davalliana*. The average value given by Hamrick & Godt (1989) for outcrossing, wind-pollinated species is $P = 49.7\%$. A similar value was found by Jonsson *et al.* (1996) who analysed genetic diversity in *Carex bigelowii*, an outcrossing, wind pollinated species. *C. davalliana* is thought to be a predominately clonal species, but Billeter (2001) found a high germination rate and seedling density of this species in the field, which suggests that sexual reproduction does contribute substantially to population dynamics.

Succisa pratensis is a dicotyledonous, outcrossing species (Adams 1954). Pollination occurs predominately by insects. The species exhibits rather high levels of genetic diversity at the population level ($P = 51.1\%$, $H_e = 0.181$) when compared to the values given by Hamrick & Godt (1989) for dicotyledons ($P = 29.0\%$, $H_e = 0.096$). When combining the traits of "dicot" and "outcrossing", average values given by Hamrick & Godt (1996; $P = 54.0$, $H_e = 0.165$) are more similar to the observed ones. Moreover, *S. pratensis* is a long-lived perennial, a trait that can further increase genetic diversity (Hamrick & Godt 1996). Values found in this study are also similar to those obtained by Hooftman (2001), i.e. $P = 52.3\%$ and $H_e = 0.184$. In com-

parison to our values, Grünbauer *et al.* (1999) found rather high H_e values ranging from 0.25 to 0.35 for *S. pratensis* in Southern Germany. However, these workers analysed isozyme variation using flowers, and all enzymes analysed were polymorphic, except for one. These methodological differences could explain the high values of H_e obtained by Grünbauer *et al.* (1999).

We did not find any significant difference in genetic variation between populations from fallows and from mown fen meadows in either species (Table 3). This negative result concurs with the findings of Gray *et al.* (1985) and of Hartnett & Bazzaz (1985). Both studies found no decline in genetic diversity of plant species during secondary succession. Hartnett & Bazzaz (1985) showed that no potential selective loss of a large number of genotypes through succession does occur in *Solidago canadensis*. It also supports the findings of Billeter (2001), who found no management-induced differences in growth and reproductive traits of both species in a common-garden experiment. Phenotypic plasticity, and not genetic change, seems to play the major role in the demographic and morphological changes observed in the field after abandonment. Bazzaz' (1996) assumption that genetic diversity decreases in the course of old field succession can not be confirmed by our study. However, old field succession commonly involves the colonisation of former agricultural fields, i.e. bare, unvegetated soils, whereas fens are characterised by high vegetation cover and high species richness. The starting point of succession therefore is an established community, and plants can hold out for a long time, keeping a long-term memory of earlier conditions, as suggested by Eriksson (1996) for remnant populations.

Habitat fragmentation, as opposed to secondary succession, had much more drastic

effects on genetic diversity. Hooftman (2001) analysed the same species for the same loci (Table 2) and found a distinct effect of habitat fragmentation on both species. Small, isolated wetlands showed a clear decline in genetic diversity, when compared to large, intact fens (Hooftman 2001).

These different results can be partially explained by the mechanisms of change. In the process of habitat fragmentation individual genets are destroyed and lost to the community right from the beginning. This lowers initial population size as well as the number of genotypes and increases the chance for inbreeding and genetic drift in the population (i.e. Menges 1990; Ellstrand & Elam 1993; Oostermeijer *et al.* 1995; Young *et al.* 1996). In the case of abandonment, however, the population size and the gene pool remain intact, at least in the beginning, and growing conditions change slowly. Therefore, species might be able to adjust growth at least partially to the changing conditions and are not likely to suffer negative effects on genetic diversity during the first decades of abandonment.

Another important factor is the time scale involved. While habitat fragmentation of many Swiss fens occurred more than a hundred years ago (Hooftman 2001), our fallows are not more than 35 years old. Both *C. davalliana* and *S. pratensis* are long-lived species and individuals can reach more than 30 years (M. Peintinger, pers. comm.). Genets can thus persist for a long time and preserve the initial genetic diversity, even if seedling recruitment is impaired. In this way, perennials like *C. davalliana* and *S. pratensis* store variation, which can become available to selection over several seasons (e.g. Jain 1979). Therefore, it is not likely that a drastic decrease in genetic diversity occurs during the first 30 to 40 years following abandonment. However, the slight decrease in all measured

variables (Table 3) suggests that genetic diversity may not be maintained indefinitely.

We conclude from the data presented here that there is no immediate threat of losing genetic variability in populations of fallows, at least initially. Moreover, Billeter (2001) could show that changes in the growth and fecundity of *C. davalliana* and *S. pratensis* observed in fallows are phenotypic and have no heritable background. The re-establishment of mowing in these meadows is desirable because traditionally managed fen meadows usually have higher species richness than fallow fens and contain many rare and endangered species (Diemer *et al.* 2001). Considering the findings of these studies, the chances of restoring the former state of an abandoned fen by re-establishment of the traditional mowing regime look rather promising, as long as species have not completely disappeared. Indeed, Billeter (2001) found that species richness of fallow fens increased rapidly following the re-establishment of mowing.

Acknowledgements

We thank D. Hooftman, D. Theis, J. Lienert and P. Kehrlı for help in the field and laboratory, B. Schmid for statistical advice, B. Roy for providing us with starch, and two referees for comments on the manuscript. A special thanks to the farmers, owners and cantonal authorities, who allowed us to work on their fens. This work was supported by a grant from the Swiss National Science Foundation (No. 31-50669.97 to M. D.).

References

- Adams, A.W. (1954) *Succisa pratensis* Moench (*Scabiosa succisa* L.). *Journal of Ecology*, **43**, 709–718.
- Bosshard, A., Andres, F., Stromeyer, S. & Wohlgemuth, Th. (1988) Wirkung einer kurzfristigen

- Brache auf das Ökosystem eines anthropogenen Kleinseggenriedes – Folgerungen für den Naturschutz. *Berichte des Geobotanischen Institutes der ETH*, **54**, 181–220.
- Billeter, R. (2001) *Secondary succession in fens: from communities to genes*. PhD Thesis, University of Zurich, Zürich.
- Billeter, R. & Diemer, M. (2000) Effects of abandonment on *Tofieldia calyculata* (Liliaceae), a common, subdominant wetland species. *Bulletin of the Geobotanical Institute ETH*, **66**, 37–46.
- BUWAL (1990) *Inventar der Flachmoore von nationaler Bedeutung*. Entwurf zur Vernehmlassung. Bundesamt für Umwelt, Wald und Landschaft, Bern.
- Diemer, M. & Pfadenhauer, J. (1987) Effect of differential defoliation on shoot growth, density and phytomass of three graminoids in calcareous fen. *Oikos*, **50**, 183–190.
- Diemer, M., Oetiker, K. & Billeter, R. (2001) Abandonment alters community composition and canopy structure of calcareous fens – a comprehensive survey of wetlands in NE Switzerland. *Applied Vegetation Science*, **4**, 237–246.
- Ellenberg, H. (1996) *Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht*. Ulmer, Stuttgart.
- Ellstrand, N.C. & Elam, D.C. (1993) Population genetic consequences of small population size: Implications for plant conservation. *Annual Review of Ecology and Systematics*, **24**, 217–242.
- Eriksson, O. (1996) Regional dynamics of plants: a review for remnant, source-sink and metapopulations. *Oikos*, **77**, 248–258.
- Gray, A.J., Stephens, D. & Ambrosen, H.E. (1985) Demographic genetics of the perennial heathland grass *Agrostis curtisii*. *Annual Report of the Institute of Terrestrial Ecology Cambridge*, 96–100.
- Grünbauer, G., Pfadenhauer, J. & Müller-Starck, G. (1999) Genetische Variation bei Wildpflanzen: Einfluss der Fragmentation auf Populationen von *Succisa pratensis*. *Verhandlungen der Gesellschaft für Ökologie*, **29**, 425–436.
- Hamrick, J.L. & Godt, M.J.W. (1989) Allozyme diversity in plant species. *Plant population genetics, breeding and germplasm resources* (eds. A.H.D. Brown, M.T. Clegg, A.L. Kahler & B.S. Weir), pp. 43–63. Sunderland, MA, Sinauer.
- Hamrick, J.L. & Godt, M.J.W. (1996) Effects of life history traits on genetic diversity in plant species. *Transactions of the Royal Society of London B*, **351**, 1291–1298.
- Hartnett, D.C. & Bazzaz, F.A. (1985) The genet and ramet population dynamics of *Solidago canadensis* in an abandoned field. *Journal of Ecology*, **73**, 407–413.
- Hooftman, D.A.P. (2001) *Habitat fragmentation in Swiss fen meadows: the case of common species*. PhD Thesis, University of Zürich, Zürich.
- Jain, S (1979) Adaptive strategies: polymorphism, plasticity, and homeostasis. *Topics in plant population biology* (eds. O.T. Solbrig, S. Jain, G.B. Johnson & P.H. Raven), pp. 160–187. Columbia University Press, New York.
- Jonsson, B.O., Jonsdottir, I.S. & Cronberg, N. (1996) Clonal diversity and allozyme variation in populations of the arctic sedge *Carex bigelowii* (Cyperaceae). *Journal of Ecology*, **84**, 449–459.
- Landolt, E. (1991) *Gefährdung der Farn- und Blütenpflanzen in der Schweiz mit gesamtschweizerischen und regionalen Artenlisten*. BUWAL, Bern.
- Lewis, P. O., and Zaykin, D. 2000. *Genetic Data Analysis: Computer program for the analysis of allelic data*. Version 1.0 (d15). <http://alleyn.eeb.uconn.edu/gda/>.
- Maas, D. (1988) Keimung und Etablierung von Streuwiesenpflanzen nach experimenteller Ansaat. *Natur und Landschaft*, **63**, 411–415.
- Menges, E.S. (1990) Population viability for an endangered plant. *Conservation Biology*, **4**, 52–62.
- Oostermeijer, J.G.B., van Eijck, M.W., van Leeuwen, N.C. & Den Nijs, J.C.M. (1995) Analysis of the relationship between allozyme heterozygosity and fitness in the rare *Gentiana pneumonanthe* L. *Journal of Evolutionary Biology*, **8**, 739–759.
- Paine, R.T. (1966) Food web complexity and species diversity. *American Naturalist*, **100**, 65–75.
- Payne, R.W., Lane, P.W., Digby, P.G.N., Harding, S.A., Leech, P.K., Morgan, G.W., Todd, A.D., Thompson, R., Tunnicliffe Wilson, G., Welham, S.J. & White, R.P. (1995) *Genstat 5, Release 3.22*
- Preiss, H. (1982) *Vegetation und Nährstoffumsatz von Flachmoorbiotopen im Raum von Bad Tölz unter der Berücksichtigung der Auswirkung von Nutzungsänderungen*. PhD Thesis, TU–München. München.
- Schopp-Guth, A., Maas, D., & Pfadenhauer, J. (1994) Influence of management on the seed production and seed bank of calcareous fen species. *Journal of Vegetation Science*, **5**, 569–578.

- Soltis, D.E., Haufler, C.H., Darrow, D.C. & Gastony, G.J. (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffer, and staining schedules. *American Fern Journal*, **73**, 9–27.
- Weeden, N.F. & Wendel, J.F. (1989) Genetics of plant isozymes. *Isozymes in plant biology* (eds. D.E. Soltis & P.S. Soltis), pp. 46–72. Dioscorides Press, Portland, Oregon.
- Wendel, J.F. & Weeden, N.F. (1989) Visualization and interpretation of plant isozymes. *Isozymes in plant biology* (eds. D.E. Soltis & P.S. Soltis), pp. 5–45. Dioscorides Press, Portland, Oregon.
- Wheeler, B.D. (1988) Species richness, species rarity and conservation evaluation of rich-fen vegetation in Lowland England and Wales. *Journal of Applied Ecology*, **25**, 331–353.
- Werth, C.R. (1991) Isozyme studies on the *Dryopteris spinulosa* complex. I: the origin of the log fern *Dryopteris celsa*. *Systematic Botany*, **16**, 446–461.
- Young, A., Boyle, T. & Brown, T. (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution*, **11**, 413–418.

Received 2 June 2002

Revised version accepted 15 July 2002

Appendix 1. List of enzymes used for electrophoresis, with abbreviations and E.C. numbers.

Name	Abbreviation	E.C. number
Aspartate aminotransferase	AAT	2.6.1.1
Aconitate hydratase	ACO	4.2.1.3
Alcohol dehydrogenase	ADH	1.1.1.1
Fructose-biphosphatase	FBP	3.1.3.11
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49
Glucose-6-phosphate isomerase	GPI	5.3.1.9
Isocitrate dehydrogenase	IDH	1.1.1.42
Malate dehydrogenase	MDH	1.1.1.37
Malic enzyme	ME	1.1.1.40
Phosphogluconate dehydrogenase	PGD	1.1.1.44
Phosphoglucomutase	PGM	5.4.2.2
Triose-phosphate isomerase	TPI	5.3.1.1