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# ISOZYME VARIABILITY OF THE WETLAND SPECIALIST *SWERTIA PERENNIS* (GENTIANACEAE) IN RELATION TO HABITAT SIZE, ISOLATION, AND PLANT FITNESS[1]

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We examined the effects of size and spatial isolation of fens on the isozyme variability of 17 populations of *Swertia perennis*. This long-lived perennial is a locally abundant fen specialist in Switzerland, where wetlands have been strongly fragmented. Isozyme variability was comparable to other outcrossing plants ($A = 1.53$, $AP_p = 2.01$, $P_p = 42.5$, $H_o = 0.113$, $H_e = 0.139$). *F* statistics indicated both inbreeding within and differentiation between populations ($F_{IS} = 0.076$, $F_{IT} = 0.194$, $F_{ST} = 0.128$), with moderate gene flow between populations ($N_em = 1.703$). Populations in small, isolated fens had reduced genetic variability and the highest within-population inbreeding coefficients ($F_{IS}$). Isozyme variability was significantly associated with vegetative fitness traits (MANOVA), and the magnitude of leaf herbivory decreased as the percentage of polymorphic loci increased. These data suggest that the reduced genetic variability of *S. perennis* in small, isolated populations may reduce plant fitness, thereby increasing susceptibility to herbivore damage. Our study also shows that habitat fragmentation can reduce the genetic variability of populations of fairly common habitat specialists, which so far have attracted less conservation attention than rare species.

**Key words:** calcareous fens; *F* statistics; genetic variability; Gentianaceae; habitat fragmentation; isozyme electrophoresis; phenotypic fitness measures; population viability; *Swertia perennis*; Switzerland.

Habitat fragmentation threatens many plant species (Young, Boyle, and Brown, 1996). Often the result of human activity, habitat fragmentation has two major consequences: populations become more isolated and they become smaller (Saunders, Hobbs, and Margules, 1991). Isolation can result in strong genetic differentiation between populations, especially between more geographically isolated ones. At the same time, fragmented populations are likely to lose genetic variability by genetic drift, which can increase the inbreeding level of populations (Lacy, 1987; Shaffer, 1987; Levin, 1988; Ellstrand and Elam, 1993). Inbreeding levels within small populations can further increase (Ellstrand and Elam, 1993) because of changed abundance or behavior of pollinators (Rathcke and Jules, 1993; Olesen and Jain, 1994). Higher inbreeding levels can reduce individual fitness and population viability via inbreeding depression and thus increase extinction risks (Young, Boyle, and Brown, 1996). The strongest inbreeding depression is expected in outcrossing plant populations of recently reduced size, whereas plants with a longer history of inbreeding may have purged some of their genetic load (Byers and Waller, 1999). Reduced genetic variability may also increase susceptibility to plant pathogens (Schmid, 1994; Ouborg, Biere, and Mudde, 2000). A positive feedback between genetic and demographic factors may even force small, isolated populations into an ''extinction vortex'' (Gilpin and Soulé, 1986). Positive correlations between genetic variability, population size, and fitness measures have been reported for a number of rare plant species (Oostermeijer, van Eijck, and den Nijs, 1994; Young, Boyle, and Brown, 1996; Fischer and Matthies, 1998; Fischer, van Kleunen, and Schmid, 2000). However, little emphasis has been placed on the effects of habitat fragmentation on once widespread species.

We examined the consequences of habitat fragmentation on the population genetics of *Swertia perennis* L. (Gentianaceae), a specialist of calcareous pre-alpine fens (*Caricion davallianae* alliance; Ellenberg, 1978). Wetlands are the habitat of many specialist species, and calcareous fens harbor nearly 50% of all endangered plant species in Switzerland (Landolt, 1991). Fens belong to the few remaining seminatural ecosystems of central Europe and were traditionally mown in late summer or extensively grazed. In Switzerland wetlands have been largely destroyed and severely fragmented (Hintermann, 1992); the overall wetland area has been reduced by 90% since 1800 (Broggi and Schlegel, 1989). Wetlands, in general, and fens, in particular, are thus well suited to investigations of the effects of habitat fragmentation.

The destruction and fragmentation of Swiss wetlands has decreased the number and size of *S. perennis* populations and increased their spatial isolation. *Swertia perennis* still grows abundantly in many calcareous fens in northeastern Switzerland; however, we found that 15 of 63 populations of *S. perennis* in this region had disappeared since 1871. Most of the local extinctions could be attributed to habitat fragmentation and some to changed land use as well (Lienert, Fischer, and Diemer, 2002). Moreover, we found that habitat fragmentation significantly reduced plant density and fitness (Lienert, Diemer, and Schmid, 2002) and that *S. perennis* is highly susceptible to inbreeding depression (J. Lienert and M. Fischer, unpublished data).

In the study reported here, we investigated whether habitat fragmentation also affects the genetic variability and popula-

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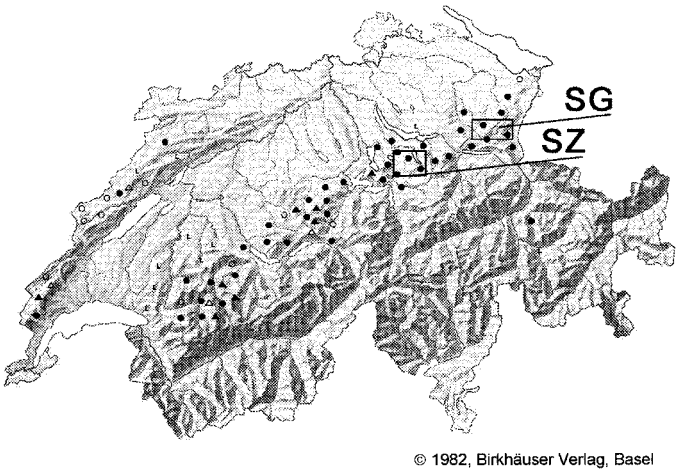


Fig. 1. Distribution of *Swertia perennis* in Switzerland (according to Welten and Sutter, 1982; map reprinted with permission from Birkhäuser Verlag, Basel) and approximate location of the two study regions (SG = St. Gallen, SZ = Schwyz). Symbols denote the occurrence of *S. perennis*: ● = in lowlands, frequent; ○ = in lowlands, rare; ▲ = in mountains, frequent; △ = in mountains, rare; L = bibliographic reference.

tion viability of *S. perennis*. To clearly distinguish between the two elements of habitat fragmentation, habitat area and spatial isolation, we studied (1) large habitats; (2) small, barely isolated habitats; and (3) small, spatially isolated habitat remnants. We assessed the genetic structure of 17 populations of *S. perennis* in northeastern Switzerland with isozyme electrophoresis. Moreover, we tested whether measures of isozyme variability were correlated with fitness measures and with the magnitude of herbivory recorded in an earlier study (Lienert, Diemer, and Schmid, 2002). We asked the following questions: (1) How large is the isozyme variability of *S. perennis*? (2) How large are population differentiation and gene flow between populations? (3) Is genetic variability reduced in small populations, especially in small, isolated populations? (4) Is genetic variability correlated with fitness measures of *S. perennis*?

## MATERIALS AND METHODS

***Study species***—*Swertia perennis* ($2n = 28$ [Hess, Landolt, and Hirzel, 1972]) has a wide but discontinuous distribution from central Europe through Asia to western North America, with a center in the European Alps (Hulten and Fries, 1986). It can be locally abundant, but is considered endangered worldwide (Jäger and Hoffmann, 1997). In Switzerland it occurs only in prealpine fens north of the Alps, in the region between the Alps and the Swiss lowlands (Fig. 1; Welten and Sutter, 1982). The Red List classification (see International Union for Conservation of Nature and Natural Resources [IUCN]: www.redlist.org) of *S. perennis* in Switzerland ranges from ''not endangered'' to ''highly endangered'' and even ''extinct'' (Landolt, 1991).

*Swertia perennis* is a long-lived, iteroparous, herbaceous perennial (Hegi, 1906). Vegetative adults have a rosette of ovate leaves and can develop daughter rosettes from lateral meristems, which are usually situated very close to the parent rosette. Reproductive shoots flower from July to August and have a single, 15–60 cm long stem with up to 30 star-shaped, protandrous flowers. The flowers are visited by various insects, including species of Coleoptera, Lepidoptera, Diptera (especially Syrphidae), and Hymenoptera (especially *Bombus* and Vespidae) (J. Lienert, personal observations). Up to 50 winged seeds develop in one ovate capsule in each flower (Hegi, 1906). Aboveground plant parts emerge in April/May, and plants die back as soon as the ground is covered with snow, typically in November.

***Study design***—The hierarchical study design comprised 18 calcareous fens, which were equally divided between two Swiss cantons, St. Gallen (SG) and Schwyz (SZ) (Hooftman et al., 1999; Fig. 1, Appendix). In each region we used three ''fen systems.'' A fen system consisted of three fens: one large Main island (>5 ha), which was accompanied by two smaller islands (<0.5 ha). The small Near island was located 40–125 m from its Main island, and the small Distant island was separated by ca. 1000 m from both its Main island and another fen. Woodlands, cattle pastures, or intensively used agricultural land separated the individual fens (Appendix). Most fens have been fragmented since at least the 1930s, according to aerial photographs (Bundesamt für Landestopographie, Wabern, Switzerland). Our study design followed Levin's (1988) assumption that the dispersal of pollen or seeds for more than 1000–2000 m is very unlikely, even over the same habitat type. Hence, the Distant islands were presumably completely isolated, whereas the Near populations were subject to sporadic gene flow. The altitude of all fens was between 900 and 1340 m above sea level, and all fens were mown annually after mid-September. Population sizes of *S. perennis* ranged from 758 to 118 500 flowering adults on the Main islands and from 7 (but with ca. 20 vegetative adults) to 6738 flowering adults on the Near and Distant islands (Appendix). One Distant fen did not contain any plants of *S. perennis*. In each fen we randomly chose five experimental plots, each $2 \times 1$ m, by using random numbers to determine the distance from the center of a fen to each of the plots. Hence, these plots represented similar areas in all populations.

***Plant material and fitness measures***—We collected leaves from 30 *S. perennis* plants in each population in August 1998. When possible, we sampled six plants per plot and preferentially selected those tagged for a study on the demography of *S. perennis* (Lienert, Diemer, and Schmid, 2002). When this was not possible or when plants had already begun to senesce, we took additional samples from randomly selected plants near the plots. To avoid collecting clones, we made sure that individual plants were separated by at least several centimeters. We stored the leaf samples for a maximum of 4 d at 4°C until further processing.

For our demographic study we assessed the number of rosette leaves, the length of the longest rosette leaf, the occurrence of herbivory of any leaves (two classes: yes/no), the magnitude of herbivory of rosette leaves (amount of leaf loss in six classes), and the aggregation of individual *S. perennis* plants (two classes: aggregated [three or more plants per 100 $cm^2$] or not aggregated). For flowering plants we also measured the number of stem leaves, the length of the longest stem leaf, the height of the flowering stem from the ground to the top of the plant (i.e., plant size), and the number of flowers per plant. We took the same measures for all plants in the study reported here, including those that were not part of the demographic study.

***Isozyme electrophoresis***—We ground each sample with 200 μL Tris-HCl grinding buffer-polyvinylpyrolidone (PVP) solution (Soltis et al., 1983), to which we added 20 μL dimethylsulfoxide (DMSO). The ground material was adsorbed onto wicks of filter paper ($2.5 \times 19$ mm), which we kept in Eppendorf tubes at −78°C for 4 mo at most.

We performed horizontal starch gel electrophoresis with 30 plants per population according to the protocols of Soltis et al. (1983) and Wendel and Weeden (1989). We used a LiOH-borate, pH 8.3 (Soltis et al., 1983) buffer system and a morpholine/citric acid, pH 6.4 (Werth, 1991) buffer system. For the latter we used 11.1 mL morpholine instead of 13.4 mL. We prepared the starch gel (12% mass/volume) according to Wendel and Weeden's (1989) protocol, but mixed the boiling buffer with an ordinary household mixer while adding the dissolved starch.

We chose the following enzyme systems for the LiOH-borate buffer: AAT, ME, NADPH, PGM, and TPI; and for the morpholine/citric acid buffer we chose: G6PDH, GDH, GPI, MDH, PGD, and SKD (Table 1). For AAT and NADPH we used the protocols of Wendel and Weeden (1989); for all others, those of Soltis et al. (1983). We modified some protocols: G6PDH without $MgCl_2$; GDH with $CaCl_2 \cdot 2H_2O$ (Wendel and Weeden, 1989); GPI and PGM with 7 μL glucose-6-phosphate dehydrogenase; PGM with 200 mg D-glucose phosphate. Staining temperature was always 40°C.

TABLE 1. Enzyme systems and distribution of the alleles in 17 populations of *Swertia perennis*. M = large Main island; N = small, barely isolated Near island; D = small, isolated Distant island. Thirteen of 22 alleles of polymorphic loci were present in every population; nine alleles occurred in fewer populations.

| Enzyme system (E.C. no.)[a] | Locus | 4° structure[b] | Allele[c] | St. Gallen[d] | | | | | | | | | Schwyz[d] | | | | | | | | | Total[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Grabs | | | Wildhaus | | | Eb.-Kappel | | | Einsiedeln | | | Unteriberg | | | Sattel | | | |
| | | | | M | N | D | M | N | D | M | N | D | M | N | D | M | N | D | M | N | D | |
| Aspartate aminotransferase (2.6.1.1) | *Aat* | d | *Aat-a* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | | | *Aat-b* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| Glucose-6-phosphate dehydrogenase (1.1.1.49) | *G6Pdh* | d | *G6Pdh-a* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 16 |
| | | | *G6Pdh-b* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| Glutamate dehydrogenase (1.4.1.2) | *Gdh1* | m | *Gdh1-a* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | | | *Gdh1-b* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | | | *Gdh1-c* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| | *Gdh2* | m | *Gdh2* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| Glucose-6-phosphate isomerase (5.3.1.9) | *Gpi1* | d | *Gpi1* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | *Gpi2* | d | *Gpi2* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| Malate dehydrogenase (1.1.1.37) | *Mdh1* | d | *Mdh1* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | *Mdh2* | d | *Mdh2* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| Malic enzyme (1.1.1.40) | *Me1* | m | *Me1* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | *Me2* | m | *Me2-a* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 16 |
| | | | *Me2-b* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | | | *Me2-c* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| NADPH dehydrogenase (1.6.99) | *Nadph* | t | *Nadph-a* | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 10 |
| | | | *Nadph-b* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | | | *Nadph-c* | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 12 |
| Phosphogluconate dehydrogenase (1.1.1.44) | *Pgd1* | m | *Pgd1-a* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | | | *Pgd1-b* | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 9 |
| | $Pgd2_a$ | d | *Pgd2-a* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | $Pgd2_b$ | | *Pgd2-b* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | | | *Pgd2-c* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 10 |
| Phosphoglucomutase (5.4.2.2) | *Pgm* | m | *Pgm-a* | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 9 |
| | | | *Pgm-b* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| Shikimate dehydrogenase (1.1.1.25) | *Skd* | m | *Skd* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| Triose-phosphate isomerase (5.3.1.1) | *Tpi1* | d | *Tpi1* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| | *Tpi2* | d | *Tpi2-a* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 |
| | | | *Tpi2-b* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 17 |
| Total number of alleles in population | | | | 28 | 26 | 25 | 28 | 27 | 26 | 25 | 25 | | 27 | 25 | 25 | 26 | 27 | 24 | 27 | 27 | 25 | |

[a] E.C. = Enzyme Commission.
[b] m = monomeric, d = dimeric, t = tetrameric.
[c] a = fast, b = slow, c = slowest.
[d] 0 = allele missing in population, 1 = allele present.
[e] Total number of populations an allele occurred in.

***Gel interpretation***—After staining, the gels were placed on a light box, photographed with a digital camera (Minolta RD-175; Minolta, Osaka, Japan), and interpreted with a computer. We interpreted banding patterns using the simplest, most conservative approach (i.e., as for a diploid species). However, even if *S. perennis* were tetraploid, possible small deviations in isozyme-variability estimates due to polyploidy should not be confounded with our sampling design.

We scored 17 loci for the 11 screened enzyme systems and found up to three alleles per locus (Table 1). Only *Pgd2* had a complicated banding pattern, which was probably caused by a duplicated locus, each with three alleles. For the analyses of genetic variability we considered *Pgd2* as two different loci; however, we did not consider *Pgd2* in calculating heterozygosities and *F* statistics. We named loci and alleles according to their electrophoretic mobility (locus $a$ = fast, $b$ = slow).

***Enzyme variability and F statistics***—To calculate standard measures of genetic variability, we used the computer program BIOSYS-1 (release 1.7; Swofford and Selander, 1989). We calculated genetic similarity between populations as Nei's (1978) unbiased identity ($I$). Moreover, we tested for correlations between genetic and corresponding geographic distances between pairs of populations with a Mantel permutation test (Mantel, 1967) in GENEPOP (version 1.2.; Raymond and Rousset, 1995). We calculated summary $F$ statistics ($F_{ST}$, $F_{IS}$, $F_{IT}$) and the estimated level of gene flow ($N_em$, under the assumptions of Wright's [1943] island model) for each polymorphic locus over all populations, and used the formula $N_em = (1 - F_{ST})/4F_{ST}$, where $N_e$ = effective population size and $m$ = migration rate. Then we calculated $F_{ST}$ for each population compared with all 16 other pooled populations, as well as $F_{IS}$, where $F_{IS} = 1 - (H_o/H_e)$, and $F_{IT}$, where $F_{IT} = F_{IS} + (1 - F_{IS})F_{ST}$ (Hartl and Clark, 1989). We also calculated $F_{ST}$ for every population relative to the other two pooled populations of the same fen system. Finally, with a chi-square test, we tested whether frequencies of homozygotes and heterozygotes per population deviated from those expected under Hardy-Weinberg equilibrium.

***Within-population genetic variability***—We analyzed among-population differences in the number of alleles per polymorphic locus ($AP_p$), percentage of polymorphic loci ($P_p$), observed heterozygosity ($H_o$), within-population inbreeding coefficient ($F_{IS}$), and genetic differentiation of populations ($F_{ST}$ for every population relative to the other two pooled populations of the same fen system) with the computer program GenStat 5 (release 3.2.; Payne et al., 1993). Using hierarchical analyses of variance (ANOVAs), we tested for the effects of the two study regions, the six fen systems (three in each region), and the three island types (Main, Near, Distant) in each fen system (test A in Table 4). To test whether the effect of island type was due to spatial isolation (test B) or to small habitat area (test C), we split the two degrees of freedom for the three island types in both possible ways. In test B we first corrected for the effect of island area by testing the Main island against the two small Near and Distant islands. Then we tested the effect of isolation as the difference between the Near island and the Distant island. In test C we first corrected for the effect of spatial isolation by testing the Main and Near islands against the Distant island. Then we tested the effect of island area as the difference between the large Main island and the small Near island. We corrected the significance levels for Type 1 error with a sequential Bonferroni test (Dunn-Šidák method; Sokal and Rohlf, 1995).

***Correlations between genetic variability and fitness***—First, we compared individual heterozygosity of vegetative adults with that of flowering adults with a one-way ANOVA. In the complementary demographic study, the following fitness measures of *S. perennis* were affected by habitat fragmentation: density of *S. perennis* (= aggregation), number and length of rosette leaves, length of the longest stem leaf, stem height, and occurrence and magnitude of leaf herbivory (Lienert, Diemer, and Schmid, 2002). Since we worked in nature reserves, we could not sample plant biomass. We therefore regarded density, herbivory, and these vegetative fitness traits as indicative of individual fitness. Usually, vegetative measures are closely correlated with reproductive fitness measures (Calvo, 1990; Schmid et al., 1994). To see whether effects of habitat fragmentation on fitness traits could have been due to effects on genetic variation, we correlated these traits with the observed heterozygosity of individual plants, with the percentage of polymorphic loci as a measure of the genetic variability of populations, and with $F_{ST}$ of each population (against the other two populations of the same fen system) as a measure of genetic differentiation between populations. We tested the relationship between the isozyme measures and all fitness measures with multiple analyses of variance (MANOVA). We corrected the significance levels of the fitness traits for Type 1 error with a sequential Bonferroni test. Here, we used the statistical software SPSS 9.0 (SPSS, Chicago, Illinois, USA).

## RESULTS

***Enzyme variability and F statistics***—Nine of the 17 loci were polymorphic (Table 1). Over all polymorphic loci we detected 22 different alleles, of which 13 were present in every population. Two alleles occurred in only two populations: *Gdh1-c* in both study regions and *Tpi2-a* within a single fen system. The *Pgd2-c* allele mainly occurred in the St. Gallen region. Of 466 different multilocus genotypes, 67 (14%) were shared by more than one individual. In 26 cases (6%), plants sharing multilocus genotypes originated from the same 1–2-m plots. These 26 cases were evenly distributed over all 17 populations. Hence, the clonality of *S. perennis* should not have influenced our results. Overall, there were 1.53 alleles per locus ($A$; Table 2) and 2.01 alleles per polymorphic locus ($AP_p$), the percentage of polymorphic loci ($P_p$) was 42.5, the observed heterozygosity ($H_o$) was 0.113, and the expected heterozygosity ($H_e$) was 0.139.

Genetic identity between populations ranged from 0.917 (Grabs Near/Sattel Distant) to 1.0 (Einsiedeln Main/Unteriberg Main; Fig. 2). Pairwise genetic distances were not correlated with geographic distances (Mantel test; $b = -0.004$, $r^2 = 0.143$, $P = 1$). Genetic differentiation between populations over all loci and populations was relatively high ($F_{ST} = 0.128$; Table 3A), which corresponds to an average gene flow between populations ($N_em$) of 1.7 individuals per generation. Per-population $F_{ST}$ against all 16 other populations was $\leq 0.110$, and against the other two populations of the same fen system, it was $\leq 0.076$ (Table 3B, C). The mean fixation index, $F_{IS}$, of individuals relative to their population was 0.076 (Table 3A), with a range of $-0.194$–$0.216$ (Table 3B). The mean overall inbreeding coefficient, $F_{IT}$, was 0.194 and ranged from $-0.182$ to 0.272 among populations.

Twenty-eight out of 101 loci that were polymorphic within populations deviated significantly from Hardy-Weinberg equilibrium (27.7%; results not shown). Of these, 82% had positive $F_{IS}$ values, indicating a heterozygote deficit. However, over all loci, no population deviated significantly from Hardy-Weinberg equilibrium.

***Within-population genetic variability***—None of the estimates of genetic variability differed between the two study regions or among the six fen systems (Table 4). The mean number of alleles per polymorphic locus ($AP_p$; $P < 0.05$; Fig. 3A) and the mean observed heterozygosity ($H_o$; $P < 0.1$; Fig. 3B) were lowest on Distant islands. Tests B and C, which tested for isolation or habitat area effects, indicated that this finding was not explained by small habitat area alone, but was also due to large isolation distance. Moreover, mean $F_{IS}$ was highest and positive on Distant islands ($P < 0.1$; Fig. 3C), whereas it was negative on Near islands and approximately zero on Main islands.

TABLE 2. Estimates of genetic variability for 17 populations of *Swertia perennis*. Standard error is given in parentheses.

| Region | Fen system | Island type | Mean sample size per locus | $A$ | $AP_p$ | $P_p$ | Mean no. polymorphic loci | $H_o$ | $H_e$ |
|---|---|---|---|---|---|---|---|---|---|
| St. Gallen | Grabs | M | 29.8 (0.2) | 1.67 (0.20) | 2.22 (0.28) | 44.4 | 6 | 0.143 (0.052) | 0.142 (0.047) |
| | | N | 26.8 (1.1) | 1.61 (0.18) | 2.00 (0.24) | 44.4 | 6 | 0.152 (0.053) | 0.144 (0.045) |
| | | D | 28.6 (1.0) | 1.50 (0.17) | 1.89 (0.26) | 38.9 | 5 | 0.107 (0.042) | 0.130 (0.047) |
| | Wildhaus | M | 29.8 (0.1) | 1.67 (0.18) | 2.22 (0.22) | 50.0 | 7 | 0.151 (0.047) | 0.166 (0.052) |
| | | N | 28.6 (0.8) | 1.61 (0.18) | 2.11 (0.26) | 44.4 | 6 | 0.143 (0.053) | 0.139 (0.053) |
| | | D | 29.7 (0.2) | 1.56 (0.17) | 2.00 (0.24) | 44.4 | 6 | 0.132 (0.050) | 0.141 (0.046) |
| | Ebnat-Kappel | M | 29.6 (0.2) | 1.44 (0.15) | 1.89 (0.26) | 38.9 | 5 | 0.115 (0.049) | 0.118 (0.045) |
| | | N | 29.7 (0.2) | 1.44 (0.15) | 1.89 (0.26) | 38.9 | 5 | 0.141 (0.062) | 0.137 (0.052) |
| | | D | | | | | | | |
| Schwyz | Einsiedeln | M | 29.6 (0.3) | 1.56 (0.17) | 2.11 (0.26) | 44.4 | 6 | 0.175 (0.063) | 0.154 (0.051) |
| | | N | 28.9 (0.9) | 1.44 (0.17) | 1.89 (0.26) | 33.3 | 5 | 0.125 (0.056) | 0.116 (0.047) |
| | | D | 28.1 (0.9) | 1.50 (0.17) | 1.89 (0.26) | 38.9 | 5 | 0.128 (0.052) | 0.132 (0.049) |
| | Unteriberg | M | 30.0 (0.0) | 1.50 (0.15) | 2.00 (0.17) | 44.4 | 7 | 0.185 (0.064) | 0.155 (0.049) |
| | | N | 29.9 (0.1) | 1.56 (0.17) | 2.11 (0.20) | 44.4 | 7 | 0.191 (0.070) | 0.165 (0.051) |
| | | D | 29.3 (0.5) | 1.44 (0.12) | 1.78 (0.15) | 44.4 | 6 | 0.142 (0.053) | 0.128 (0.043) |
| | Sattel | M | 29.5 (0.4) | 1.56 (0.15) | 2.11 (0.20) | 50.0 | 7 | 0.134 (0.050) | 0.171 (0.054) |
| | | N | 29.7 (0.3) | 1.56 (0.17) | 2.11 (0.20) | 44.4 | 7 | 0.134 (0.050) | 0.132 (0.047) |
| | | D | 27.4 (1.2) | 1.44 (0.17) | 1.89 (0.26) | 33.3 | 5 | 0.118 (0.054) | 0.132 (0.053) |
| | Means[a] | | 29.1 (0.23) | 1.53 (0.02) | 2.01 (0.03) | 42.5 | 5.9 | 0.113 (0.044) | 0.139 (0.051) |

*Note:* M = large Main island; N = small, barely isolated Near island; D = small, isolated Distant island; $A$ = mean number of alleles per locus; $AP_p$ = mean number of alleles per polymorphic locus; $P_p$ = percentage of polymorphic loci (a locus was considered polymorphic if the frequency of the most common allele was ≤0.99); $H_o$ = mean observed heterozygosity; $H_e$ = mean expected heterozygosity.

[a] Means (± 1 SE) over all populations, except for $H_o$ and $H_e$, obtained from pooled data of all populations.

***Correlations between genetic variability and fitness***—The observed heterozygosity did not differ between vegetative and flowering adults (Pearson's correlation: $N = 462$, $r = 0.01$, $P = 0.755$). Overall, herbivory was strong: 52% of plants were affected. Of these, 71% were in herbivory classes 1 and 2 (≤10% loss of total leaf area), and 29% had >10% leaf-area loss. The occurrence of herbivory was highly correlated with the magnitude of herbivory (Pearson's chi-square value: 387.14, $N = 540$, $P < 0.001$); the length of the stem leaf was highly correlated with both the length of rosette leaf and the stem height (Pearson's correlation: $N = 201$ and 203, $r = 0.409$ and 0.577, respectively, $P < 0.001$); and the number of flowers was highly correlated with stem height ($N = 203$, $r = 0.504$, $P < 0.001$). Therefore, to avoid problems due to collinearity among variates, we dismissed the occurrence of herbivory, length of stem leaf, and number of flowers from our analysis. The observed heterozygosity of individuals ($H_o$) increased marginally significantly with increasing length of the longest rosette leaf ($P < 0.1$; Table 5A, Fig. 4A) and was significantly associated with all fitness traits (MANOVA; $P < 0.01$). On the population level, the percentage of polymorphic loci and $F_{ST}$ were significantly associated with all fitness traits (MANOVA; both $P < 0.05$; Table 5B). The magnitude of herbivory decreased with increasing percentage of polymorphic loci ($P < 0.05$; Fig. 4B) and increased slightly with increasing differentiation between populations of the same fen system ($F_{ST}$; $P < 0.1$; Fig. 4C). Mean herbivory was low in all but one Main population, but increased with increasing $F_{ST}$ in Near and Distant populations (Fig. 4C).

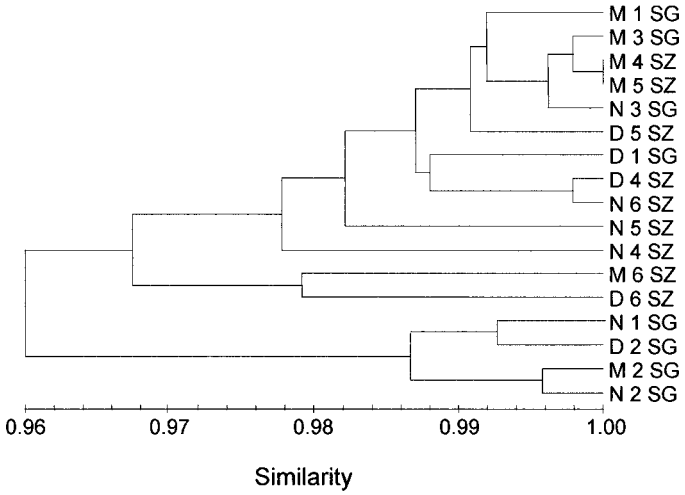


Fig. 2. Isozyme-based unweighted pair group method (UPGM) dendrogram for 17 studied populations of *Swertia perennis*, based on Nei's genetic identity (*I*). M = Main, N = Near, D = Distant. See Appendix for other abbreviations and for attributes of study sites.

## DISCUSSION

***Enzyme variability and differentiation among populations***—Enzyme variability of *S. perennis* roughly followed expectations for outbreeding plants, with over 50% polymorphic loci, only two rare alleles (*Gdh1-c*, *Tpi2-a*), and only 14% shared multilocus genotypes. Mean genetic variability of *S. perennis* was lower than the means of other outcrossing, animal-pollinated plants. In a review by Hamrick and Godt (1990), 172 outcrossing, animal-pollinated plant species had mean (±1 SE) $A$ values of 2.0 (±0.07; *S. perennis*: 1.53), $P_p$ was 50.1 (±2.0; *S. perennis*: 42.5), and $H_e$ was 0.17 (±0.01; *S. perennis*: 0.14).

Genetic differentiation between populations of *S. perennis* ($F_{ST} = 0.128$) can be compared with the genetic differentiation of other plants measured as $G_{ST}$ because $F_{ST}$ approximately equals $G_{ST}$ when there are only two alleles per locus (Hartl and Clark, 1989; Swofford and Selander, 1989). The case of *S. perennis* was similar to the one of other outcrossing plants: mean $G_{ST}$ of 76 predominantly outcrossed plants was 0.118 (±0.036); of 146 sexually reproducing plants, 0.300 (±0.087;

TABLE 3. *F* statistics and average gene flow ($N_em$, under the assumptions of Wright's island model) for *Swertia perennis*. (A) Per polymorphic locus and over all loci. (B) Genetic differentiation between each population compared with all 16 other pooled populations ($F_{ST}$), mean fixation index of individuals relative to their population ($F_{IS}$), and mean overall inbreeding coefficient of an individual ($F_{IT}$). (C) $F_{ST}$ for every population compared with the two other populations within the same fen system. Only one correlation was possible for Ebnat-Kappel, because *S. perennis* is absent from this Distant island.

| A) Locus | $F_{ST}$ | $F_{IS}$ | $F_{IT}$ | $N_em$ |
|---|---|---|---|---|
| *Aat* | 0.064 | −0.162 | −0.087 | 3.656 |
| *G6Pdh* | 0.067 | 0.150 | 0.207 | 3.481 |
| *Gdh1* | 0.141 | 0.274 | 0.377 | 1.523 |
| *Me2* | 0.117 | 0.084 | 0.191 | 1.887 |
| *Nadph* | 0.257 | 0.131 | 0.355 | 0.723 |
| *Pgd1* | 0.179 | 0.119 | 0.277 | 1.147 |
| *Pgm* | 0.029 | −0.051 | −0.021 | 8.371 |
| *Tpi2* | 0.030 | 0.483 | 0.498 | 8.083 |
| All loci | 0.128 | 0.076 | 0.194 | 1.703 |

| B) Fen system | Island type | $F_{ST}$ | $F_{IS}$ | $F_{IT}$ |
|---|---|---|---|---|
| Grabs | Main | 0.016 | −0.007 | 0.009 |
| | Near | 0.056 | −0.056 | 0.004 |
| | Distant | 0.029 | 0.177 | 0.201 |
| Wildhaus | Main | 0.041 | 0.090 | 0.128 |
| | Near | 0.025 | −0.029 | −0.003 |
| | Distant | 0.033 | 0.064 | 0.095 |
| Ebnat-Kappel | Main | 0.018 | 0.025 | 0.043 |
| | Near | 0.018 | −0.029 | −0.011 |
| | Distant | — | — | — |
| Einsiedeln | Main | 0.005 | −0.136 | −0.131 |
| | Near | 0.060 | −0.078 | −0.013 |
| | Distant | 0.018 | 0.030 | 0.048 |
| Unteriberg | Main | 0.010 | −0.194 | −0.182 |
| | Near | 0.038 | −0.158 | −0.114 |
| | Distant | 0.035 | −0.109 | −0.071 |
| Sattel | Main | 0.071 | 0.216 | 0.272 |
| | Near | 0.040 | −0.015 | 0.025 |
| | Distant | 0.110 | 0.106 | 0.204 |

| | $F_{ST}$ | | |
|---|---|---|---|
| C) Fen system | Main vs. (Near + Distant) | Near vs. (Main + Distant) | Distant vs. (Main + Near) |
| Grabs | 0.049 | 0.055 | 0.047 |
| Wildhaus | 0.031 | 0.018 | 0.042 |
| Ebnat-Kappel | 0.024 | 0.024 | — |
| Einsiedeln | 0.017 | 0.069 | 0.035 |
| Unteriberg | 0.015 | 0.025 | 0.036 |
| Sattel | 0.037 | 0.052 | 0.076 |

Loveless and Hamrick, 1984), and of 124 outcrossing, animal-pollinated plants, 0.197 (Hamrick and Godt, 1990). However, $F_{ST}$ of *S. perennis* was high compared with other long-lived plants (mean $G_{ST}$ of 48 long-lived plants = 0.077 [±0.027] and mean $G_{ST}$ of 43 outcrossing, long-lived perennial plants = 0.068 [Loveless and Hamrick, 1984]). Ellstrand and Elam (1993) considered $G_{ST}$ values of >0.1 to represent high among-population variation. Generally, $F_{ST}$ values of 0.05–0.15 indicate moderate genetic differentiation (Hartl, 1988). Given the perennial and outcrossing life history of *S. perennis*, we conclude that genetic differentiation between populations was at least as high as, if not higher than, expected.

Geographic and genetic distances between pairs of populations were not correlated, which follows the distribution of alleles (Table 1). Neither the populations of the two study regions nor those of the same fen system were grouped in clusters (but see Wildhaus Main/Near and Sattel Main/Distant in Fig. 2). Hence, gene flow between geographically close populations (i.e., within fen systems) was not higher than between geographically more distant ones.

TABLE 4. Results of the hierarchical analyses of variance (ANOVAs) with significant outcomes of the mean estimates of genetic variability of *Swertia perennis*. "Region" denotes the two study regions, and "System" denotes the six fen systems (three in each region), each consisting of a large Main island, a small, barely isolated Near island, and a small, isolated Distant island. Test A tests for differences between the three island types (Main, Near, Distant). Tests B and C are more-detailed tests on the island level. Test B tests for isolation effects between the two small islands (Near and Distant) after effects of habitat area were removed, and C tests for habitat-area effects between the barely isolated Main and Near islands after isolation effects were removed (see MATERIALS AND METHODS).

| Sources of variation | | df | $AP_p$ | $H_o$ | $F_{IS}$ |
|---|---|---|---|---|---|
| Region | | 1 | 0.0067 | 0.00066 | 0.0190 |
| System | | 4 | 0.0173 | 0.00082 | 0.0259 |
| Island | Test A | 2 | 0.0734** | 0.00131 | 0.0189 |
| | | (1) | 0.089** | 0.00079 | 0.0002 |
| | Test B | (1) | 0.0679** | 0.00183* | 0.0376* |
| | | (1) | 0.1307*** | 0.00259** | 0.0270 |
| | Test C | (1) | 0.0161 | 0.00002 | 0.0108 |
| Residuals | | 9 | 0.0061 | 0.00025 | 0.0052 |

*Note:* $AP_p$ = mean number of alleles per polymorphic locus, $H_o$ = mean observed heterozygosity, $F_{IS}$ = fixation index of individuals relative to their population. Significance levels were corrected with a sequential Bonferroni test (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$).

The $F_{ST}$ value of 0.128 corresponds to a gene flow ($N_em$) of 1.7 migrants per generation (under the assumptions of Wright's island model, but see Whitlock and McCauley, 1999). However, even 5–20 migrants per generation may not prevent the loss of genetic diversity within and differentiation between populations (Lacy, 1987). Furthermore, the measured $F_{ST}$ values of the long-lived *S. perennis* may still partly reflect the situation of the prefragmentation landscape. Today, gene flow is presumably even smaller than our estimate. Hence, it is possible that gene flow between populations of *S. perennis* is insufficient to counteract the negative effects of habitat fragmentation on genetic diversity.

***Genetic variability and inbreeding within populations***—Our most important finding is the following: only the combination of geographic isolation and small population size reduced some genetic variability measures of *S. perennis* populations (Table 4, test B; Fig. 3A, B). Small population size alone did not significantly reduce genetic variability in Near islands, which are close to large islands, and therefore presumably experience much greater gene flow (Table 4, test C). Hence, rare long-distance dispersal to Distant islands is probably insufficient to counteract the negative effects of small population size.

Decreased isozyme variability in smaller populations has been reported in other studies. The number of alleles per locus (*A*) and the percentage of polymorphic loci ($P_p$) were most often positively correlated with population size in 10 rare or endemic plant species (Ellstrand and Elam, 1993) and in 11 of 16 woody species and perennial herbs (Frankham, 1996). The same relationship was found for *Salvia pratensis* L., *Scabiosa columbaria* L. (van Treuren et al., 1991), *Gentiana*

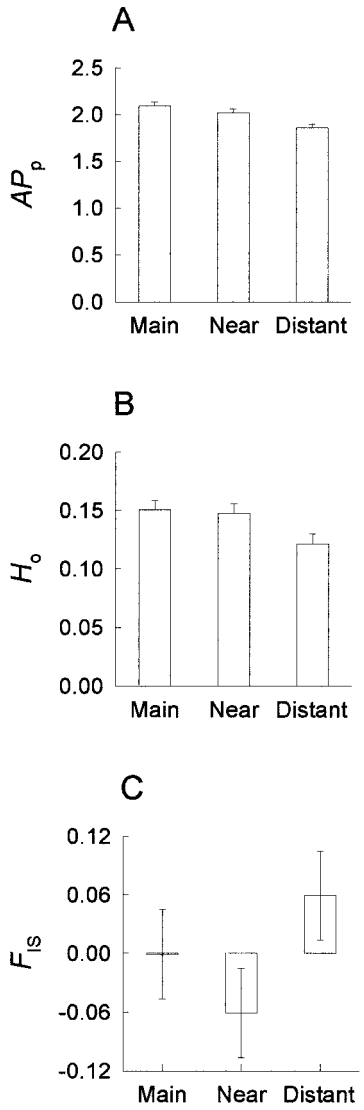


Fig. 3. Measures of isozyme variability of *Swertia perennis* on the six large Main, six small, barely isolated Near, and five small, isolated Distant habitat islands. Depicted relationships were (nearly) significant regarding isolation distance, but not regarding island area alone (see Table 4). A. Mean number of alleles per polymorphic locus ($AP_p$; $P < 0.05$). B. Mean observed heterozygosity ($H_o$; $P < 0.1$). C. Fixation index of individuals relative to their population ($F_{IS}$; $P < 0.1$). Error bars denote 1 SE.

*pneumonanthe* L. (Raijmann et al., 1994), *Spiranthes sinensis* (Pers.) Ames (Sun, 1996), and *Rutidosis leptorrhynchoides* F. Muell. (Young, Brown, and Zich, 1999). Like *S. perennis*, these species have become increasingly rare because of habitat fragmentation and destruction. However, population sizes in these studies were often much smaller than ours, the majority of populations often harboring ≤100 flowering adults (e.g., *S. pratensis*, *G. pneumonanthe*, *S. sinensis*). Moreover, these species are considered rare or endangered in the study regions, although this is not yet the case for *S. perennis*.

Reports of positive correlations between population size and heterozygosity ($H_e$ or $H_o$) are much rarer than those for $A$ or $P_p$ (Ellstrand and Elam, 1993; Frankham, 1996; but see Godt, Johnson, and Hamrick, 1996 and Luijten et al., 2000). In our study, observed heterozygosity ($H_o$) was also nearly significantly reduced on Distant islands, which indicates relatively pronounced effects of habitat fragmentation on the genetic variability of individual plants. Again, this is especially interesting because population sizes on Distant islands ranged from 7 to 4446 flowering adults (Appendix). Clonal reproduction of *S. perennis* cannot account for this reduced genetic variability, because the 6% of plants that could possibly have been clonal were evenly distributed among populations (see RESULTS). Hence, genetic variability is already reduced in even fairly large, isolated populations. The largest Distant population (Unteriberg) was larger than its Main population because of the high density of *S. perennis* (Appendix). Nevertheless, within the fen system of Unteriberg, genetic variability measures were still highest on the Main and Near islands (Table 2), although the Distant population had an excess of heterozygotes (see below).

Inbreeding was presumably responsible for the reduced genetic variability in *S. perennis* populations, because within-population inbreeding estimated over all populations ($F_{IS}$ = 0.076), and especially of individual populations ($F_{IS}$ = −0.194–0.216), was relatively high. The $F_{IS}$ of outcrossing plants with random mating within populations is expected to be near zero (Hartl and Clark, 1989). Moreover, $F_{IS}$ was nearly significantly higher on Distant islands in the ANOVA (Table 4, Fig. 3C). Additionally, genetic drift or subsampling may have caused population differentiation (mean $F_{ST}$ = 0.128) and contributed to the high total amount of inbreeding ($F_{IT}$ = 0.194). Further, Table 3B shows that all but one of the five Distant populations (the unusually large one in Unteriberg, with 4446 flowering adults) had a heterozygote deficit ($F_{IS} > 0$), whereas all Near populations had a nonsignificant excess of heterozygotes ($F_{IS} < 0$; Fig. 3C). Increased $F_{IS}$ values can be caused both by inbreeding (Charlesworth and Charlesworth, 1987) and by structuring within sample units (Wahlund effect; e.g., Hartl and Clark, 1989). However, since our sampling procedure was randomized, and since sample areas were similar in all island types, inbreeding in Distant islands is a more likely explanation. The negative $F_{IS}$ values on Near islands could still reflect their historical association with the corresponding Main islands. Inbreeding may also be attributed to less effective pollination in small habitats, which increases selfing mechanisms (e.g., attraction of fewer insects [Sih and Baltus, 1987] and altered or ineffective pollinator behavior [Heinrich, 1979; Groom, 1998]). The Sattel Main island had an unexpectedly high $F_{IS}$ value (0.216). A possible explanation is that pollinator flight distances were shorter by chance, so that less migrant pollen contributed to the total pollen pool in this population (Richards, Church, and McCauley, 1999).

Most previous studies have focused only on population size, implicitly assuming the same degree of isolation for all populations. As we did in our study, Hall, Walker, and Bawa (1996) found a negative relationship between genetic variability and geographic isolation for the tree *Pithecellobium elegans* Ducke. This was not the case for *Silene regia* Sims (Dolan, 1994), possibly because of effective long-distance pollen dispersal by hummingbirds. In the forest tree *Acer saccharum* Marsh. gene flow between populations actually increased after fragmentation, presumably because of enhanced wind flux after fragmentation (Foré et al., 1992). Likewise, *Rutidosis leptorrhynchoides* did not show an isolation effect; the observed levels of genetic differentiation may represent the prefragmentation situation (Young, Brown, and Zich, 1999). This could also be true for *S. perennis*; hence, the neg-

TABLE 5. Relationship between isozyme variability and fitness traits of *Swertia perennis*. (A) Pearson's correlation between observed heterozygosity of individuals ($H_o$) and fitness traits of individuals, and multiple analysis of variance (MANOVA) of the relationship between $H_o$ and all fitness traits. (B) Pearson's correlation between the mean fitness traits of populations and (1) the percentage of polymorphic loci of populations ($P_p$) and (2) the genetic differentiation of populations (measured as $F_{ST}$ of each population against the other two populations of the same fen system); and MANOVAs of the relationship between these two population genetic measures and all fitness traits. The significance levels of fitness traits were corrected with a sequential Bonferroni test (*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$).

| | | | Observed heterozygosity of individuals ($H_o$) | | | |
|---|---|---|---|---|---|---|
| A) Fitness traits of individuals | df | *N* | *r* | *F* | | |
| Aggregation | | 416 | 0.087 | | | |
| No. rosette leaves, vegetative adults | | 253 | 0.108 | | | |
| Length of longest rosette leaf, veg. adults | | 252 | 0.157* | | | |
| Length of flowering stem | | 204 | 0.139 | | | |
| Magnitude of herbivory, rosette leaves | | 508 | −0.081 | | | |
| MANOVA of all fitness traits | 5, 75 | | | 3.910*** | | |
| | | | Percentage of polymorphic loci ($P_p$) | | $F_{ST}$ of each pop. vs. two in same system | |
| B) Mean fitness traits of populations | df | *N* | *r* | *F* | *r* | *F* |
| Aggregation | | 17 | −0.042 | | −0.393 | |
| No. rosette leaves, all plants | | 17 | 0.451 | | −0.162 | |
| Length of longest rosette leaf, all plants | | 17 | −0.091 | | −0.381 | |
| Length of flowering stem | | 17 | 0.451 | | −0.083 | |
| Magnitude of herbivory, rosette leaves | | 17 | −0.639** | | 0.586* | |
| MANOVA of all fitness traits | 5, 75 | | | 4.431** | | 5.282** |

ative effects of fragmentation on genetic diversity could increase in the future. To obtain a more general picture of the relative importance of population size and isolation, we suggest that geographic isolation be considered in future studies of genetic variability of populations.

***Correlations between genetic variability and fitness***—Isozyme variability of *S. perennis* was positively correlated with all vegetative fitness traits (but only weakly with single fitness traits) and negatively correlated with the magnitude of herbivory (Table 5, Fig. 4). Because reduced leaf size or vegetative plant mass can lead to reduced reproductive success (e.g., Calvo, 1990; Schmid et al., 1994), long-term viability of populations with reduced genetic variability could become destabilized. Indications for positive relationships between population size and genetic variability on the one hand and/or between genetic variability and fitness on the other were found in *Gentiana pneumonanthe* (Oostermeijer, van Eijck, and den Nijs, 1994; Oostermeijer et al., 1995), *Gentianella germanica* (Willdenow) Börner (Fischer and Matthies, 1998), *Pedicularis palustris* L. (Schmidt and Jensen, 2000), *Rutidosis leptorrhynchoides* (Young et al., 2000), and *Ranunculus reptans* L. (Fischer, van Kleunen, and Schmid, 2000), but not in *Salvia pratensis* (Ouborg and van Treuren, 1994), *Lychnis flos-cuculi* L. (Hauser and Loeschcke, 1994), and *Arnica montana* L. (Luijten et al., 2000). In *L. flos-cuculi* only one of four examined populations was small (300 ramets; Hauser and Loeschcke, 1994), and the self-incompatibility system of *A. montana* may have reduced the importance of inbreeding depression (Luijten et al., 2000). All of the above species except *L. flos-cuculi* are regarded as rare or endangered in the study regions, and their population sizes were often much smaller than those of *S. perennis* (exceptions are *R. leptorrhynchoides* and *L. flos-cuculi*). Therefore, it can be expected that inbreeding effects are stronger than in our fairly large study populations. Ouborg and van Treuren (1994) suggest that small populations of *S. pratensis* could be in an early phase of genetic erosion, where the supposed selectively neutral variation of isozymes is already reduced in small populations, but selectively nonneutral variation is only slightly affected. Similarly, isozyme variability of the long-lived *S. perennis* may not be very representative for variation at fitness loci, which could account for the rather weak correlation of genetic variability with fitness measures in our study.

Increased herbivory in small and genetically less diverse populations may have genetic but also nongenetic causes. Small sites with species-rich vegetation could attract a greater diversity of invertebrates than the surrounding agricultural land (Wettstein and Schmid, 1999). Moreover, higher plant fitness could be due to better habitat quality in large field sites than in small ones. It is possible that nutrient influx from the surrounding farmland into the smallest fens was increased because of an unfavorable edge-to-perimeter relationship. In contrast, nutrient influx in large fens may be buffered at the edges and not affect the center.

Nevertheless, while nutrient influx could have contributed to reduced fitness in smaller islands, it cannot explain the significant differences in genetic variability between the similarly small Near and Distant sites (Fig. 3) or the positive relationship between genetic differentiation and the magnitude of herbivory in all small sites (Fig. 4C). We conclude that reduced genetic variability may have contributed to reduced fitness of *S. perennis* in small and isolated populations, but that environmental influences presumably also adversely affect these populations. The observed genetic effects of small population size and isolation suggest that phenotypic effects of reduced genetic variability may become increasingly important for the long-term viability of this species.

***Conservation implications***—*Swertia perennis*, a habitat specialist, is negatively affected by habitat fragmentation, and spatial isolation of about 1000 m is sufficient to reduce the genetic variability of small populations of this species. So far, formerly common habitat specialists that, like *S. perennis*, are still growing abundantly on many sites have attracted far less conservation attention than rare species. However, if more common plant species decline in increasingly fragmented landscapes, rarer species that depend on common ones will inev-

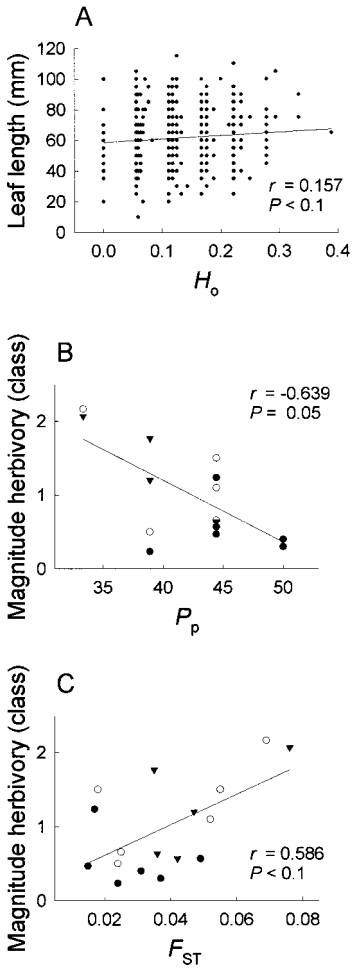


Fig. 4. Correlations between genetic variability and fitness traits of *Swertia perennis*. A. Correlation between individual observed heterozygosity ($H_o$) with the length of the longest rosette leaf of vegetative adults. B. Correlation between population means of the percentage of polymorphic loci ($P_p$) with the magnitude of mean herbivory of rosette leaves (class 0: none; class 1: ≤5% of total leaf area; class 2: 5–10%; class 3: 10–25%). C. Correlation between means of genetic differentiation of populations ($F_{ST}$ of every population relative to the other two populations of the same fen system) with the magnitude of mean herbivory of rosette leaves. Habitat islands: ● = large Main island; ○ = small, barely isolated Near island; ▼ = small, isolated Distant island.

itably become more threatened. We therefore recommend that future investigations and conservation activities consider the importance of more common plants in preserving biodiversity.

## LITERATURE CITED


BROGGI, M. F., AND H. SCHLEGEL. 1989. Mindestbedarf an naturnahen Flächen in der Kulturlandschaft. Nationales Forschungsprogramm ''Nutzung des Bodens in der Schweiz,'' Bericht 31. Liebefeld-Bern, Switzerland.

BYERS, D. L., AND D. M. WALLER. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics* 30: 479–513.

CALVO, R. N. 1990. Four-year growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae) in South Florida. *American Journal of Botany* 77: 736–741.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.

DOLAN, R. W. 1994. Patterns of isozyme variation in relation to population size, isolation, and phytogeographic history in royal catchfly (*Silene regia*; Caryophyllaceae). *American Journal of Botany* 81: 965–972.

ELLENBERG, H. 1978. Vegetation Mitteleuropas mit den Alpen in ökologischer Sicht. Ulmer, Stuttgart, Germany.

ELLSTRAND, N. C., AND D. R. ELAM. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217–242.

FISCHER, M., AND D. MATTHIES. 1998. RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). *American Journal of Botany* 85: 811–819.

FISCHER, M., M. VAN KLEUNEN, AND B. SCHMID. 2000. Genetic allee effects on performance, plasticity and developmental stability in a clonal plant. *Ecology Letters* 3: 530–539.

FORÉ, S. A., R. J. HICKEY, J. L. VANKAT, S. I. GUTTMAN, AND R. L. SCHAEFER. 1992. Genetic structure after forest fragmentation: a landscape ecology perspective on *Acer saccharum*. *Canadian Journal of Botany* 70: 1659–1668.

FRANKHAM, R. 1996. Relationship of genetic variation to population size in wildlife. *Conservation Biology* 10: 1500–1508.

GILPIN, M. E., AND M. E. SOULÉ. 1986. Minimum viable populations: processes of species extinction. *In* M. E. Soulé [ed.], Conservation biology: the science of scarcity and diversity, 19–34. Sinauer, Sunderland, Massachusetts, USA.

GODT, M. J. W., B. R. JOHNSON, AND J. L. HAMRICK. 1996. Genetic diversity and population size in four rare southern Appalachian plant species. *Conservation Biology* 10: 796–805.

GROOM, M. J. 1998. Allee effects limit population viability of an annual plant. *American Naturalist* 151: 487–496.

HALL, P., S. WALKER, AND K. BAWA. 1996. Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. *Conservation Biology* 10: 757–768.

HAMRICK, J. L., AND M. J. W. GODT. 1990. Allozyme diversity in plant species. *In* A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir [eds.], Plant population genetics, breeding, and genetic resources, 43–63. Sinauer, Sunderland, Massachusetts, USA.

HARTL, D. L. 1988. A primer of population genetics, 2nd ed. Sinauer, Sunderland, Massachusetts, USA.

HARTL, D. L., AND A. G. CLARK. 1989. Principles of population genetics, 2nd ed. Sinauer, Sunderland, Massachusetts, USA.

HAUSER, T. P., AND V. LOESCHCKE. 1994. Inbreeding depression and mating-distance dependent offspring fitness in large and small populations of *Lychnis flos-cuculi* (Caryophyllaceae). *Journal of Evolutionary Biology* 7: 609–622.

HEGI, G. 1906. Illustrierte Flora von Mitteleuropa, vol. 3, *Swertia*, 1973–1976. J. F. Lehmanns, Munich, Germany.

HEINRICH, B. 1979. Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia* 40: 235–245.

HESS, H. E., E. LANDOLT, AND R. HIRZEL. 1972. Flora der Schweiz, vol. 3, Plumbaginaceae–Compositae, 18–19. Birkhäuser, Basel, Switzerland.

HINTERMANN, U. 1992. Inventar der Moorlandschaften von besonderer Schönheit und von nationaler Bedeutung. Schlussbericht, Schriftenreihe Umwelt Nummer 168. BUWAL (Bundesamt für Umwelt, Wald und Landschaft), Bern, Switzerland.

HOOFTMAN, D. A. P., M. DIEMER, J. LIENERT, AND B. SCHMID. 1999. Does habitat fragmentation reduce the long-term survival of isolated populations of dominant plants? A field design. *Bulletin of the Geobotanical Institute ETH* 65: 59–72.

HULTEN, E., AND M. FRIES. 1986. Atlas of North European vascular plants, north of the tropic of cancer. Koeltz, Königstein, Germany.

JÄGER, E. J., AND M. H. HOFFMANN. 1997. Schutzwürdigkeit von Gefässpflanzen aus der Sicht der Gesamtareale. *Zeitschrift für Ökologie und Naturschutz* 6: 225–232.

LACY, R. C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology* 1: 143–158.

LANDOLT, E. 1991. Gefährdung der Farn- und Blütenpflanzen in der Schweiz mit gesamtschweizerischen und regionalen roten Listen. BUWAL (Bundesamt für Umwelt, Wald und Landschaft), Bern, Switzerland.

LEVIN, D. A. 1988. Consequences of stochastic elements in plant migration. *American Naturalist* 132: 643–651.

LIENERT, J., M. DIEMER, AND B. SCHMID. 2002. Effects of habitat fragmentation on population structure and fitness components of the wetland

specialist *Swertia perennis* L. (Gentianaceae). *Basic and Applied Ecology* 3, in press.

LIENERT, J., M. FISCHER, AND M. DIEMER. 2002. Local extinctions of the wetland specialist *Swertia perennis* L. (Gentianaceae) in Switzerland: a revisitation study based on herbarium records. *Biological Conservation* 103/1: 65–76.

LOVELESS, M. D., AND J. L. HAMRICK. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.

LUIJTEN, S. H., A. DIERICK, J. G. B. OOSTERMEIJER, L. E. L. RAIJMANN, AND H. C. M. DEN NIJS. 2000. Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in the Netherlands. *Conservation Biology* 14: 1776–1787.

MANTEL, N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Research* 27: 209–220.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

OLESEN, J. M., AND S. K. JAIN. 1994. D: Fragmented plant populations and their lost interactions. *In* V. Loeschcke, J. Tomiuk, and S. K. Jain [eds.], Conservation genetics, 417–426. Birkhäuser, Basel, Switzerland.

OOSTERMEIJER, J. G. B., M. W. VAN EIJCK, AND J. C. M. DEN NIJS. 1994. Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). *Oecologia* 97: 289–296.

OOSTERMEIJER, J. G. B., M. W. VAN EIJCK, N. C. VAN LEEUWEN, AND J. C. M. DEN NIJS. 1995. Analysis of the relationship between allozyme heterozygosity and fitness in the rare *Gentiana pneumonanthe* L. *Journal of Evolutionary Biology* 8: 739–759.

OUBORG, N. J., A. BIERE, AND C. L. MUDDE. 2000. Inbreeding effects on resistance and transmission-related traits in the *Silene-Microbotryum* pathosystem. *Ecology* 81: 520–531.

OUBORG, N. J., AND R. VAN TREUREN. 1994. The significance of genetic erosion in the process of extinction. IV. Inbreeding load and heterosis in relation to population size in the mint *Salvia pratensis*. *Evolution* 48: 996–1008.

PAYNE, R. W., P. W. LANE, P. G. N. DIGBY, S. A. HARDING, P. K. LEECH, G. W. MORGAN, A. D. TODD, R. THOMPSON, G. TUNICLIFFE WILSON, S. J. WELHAM, AND R. P. WHITE. 1993. GenStat 5 reference manual. Clarendon Press, Oxford, UK.

RAIJMANN, L. E. L., N. C. VAN LEEUWEN, R. KERSTEN, J. G. B. OOSTERMEIJER, H. C. M. DEN NIJS, AND S. B. J. MENKEN. 1994. Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. *Conservation Biology* 8: 1014–1026.

RATHCKE, B. J., AND E. S. JULES. 1993. Habitat fragmentation and plant–pollinator interactions. *Current Science* 65: 273–277.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2.): population genetics software for exact tests and ecuminicism. *Journal of Heredity* 86: 248–249.

RICHARDS, C. M., S. CHURCH, AND D. E. MCCAULEY. 1999. The influence of population size and isolation on gene flow by pollen in *Silene alba*. *Evolution* 53: 63–73.

SAUNDERS, D. A., R. J. HOBBS, AND C. R. MARGULES. 1991. Biological consequences of ecosystem fragmentation: a review. *Conservation Biology* 5: 18–32.

SCHMID, B. 1994. Effects of genetic diversity in experimental stands of *Solidago altissima*: evidence for the potential role of pathogens as selective agents in plant populations. *Journal of Ecology* 82: 165–175.

SCHMID, B., W. POLASEK, J. WEINER, A. KRAUSE, AND P. STOLL. 1994. Modeling of discontinuous relationships in biology with censored regression. *American Naturalist* 143: 494–507.

SCHMIDT, K., AND K. JENSEN. 2000. Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (Scrophulariaceae) and its relation to population size and reproductive components. *American Journal of Botany* 87: 678–689.

SHAFFER, M. 1987. Minimum viable populations: coping with uncertainty. *In* M. E. Soulé [ed.], Viable populations for conservation, 69–86. Cambridge University Press, Cambridge, UK.

SIH, A., AND M. BALTUS. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. *Ecology* 68: 1679–1690.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry: the principles and practice of statistics in biological research, 3rd ed. W. H. Freeman, New York, New York, USA.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, AND G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffer, and staining schedules. *American Fern Journal* 73: 9–27.

SUN, M. 1996. Effects of population size, mating system, and evolutionary origin on genetic diversity in *Spiranthes sinensis* and *S. hongkongensis*. *Conservation Biology* 10: 785–795.

SWOFFORD, D. L., AND R. B. SELANDER. 1989. BIOSYS-1: a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Illinois Natural History Survey, Champaign, Illinois, USA.

VAN TREUREN, R., R. BIJLSMA, W. VAN DELDEN, AND N. J. OUBORG. 1991. The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity* 66: 181–189.

WELTEN, M., AND H. C. R. SUTTER. 1982. Verbreitungsatlas der Farn- und Blütenpflanzen der Schweiz, vol. 2. Birkhäuser, Basel, Switzerland.

WENDEL, J. F, AND N. F. WEEDEN. 1989. Visualization and interpretation of plant isozymes. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 5–45. Dioscorides Press, Portland, Oregon, USA.

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.

WETTSTEIN, W., AND B. SCHMID. 1999. Conservation of arthropod diversity in montane wetlands: effect of altitude, habitat quality and habitat fragmentation on butterflies and grasshoppers. *Journal of Applied Ecology* 36: 363–373.

WHITLOCK, M. C., AND D. E. MCCAULEY. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity* 82: 117–125.

WRIGHT, S. 1943. Isolation by distance. *Genetics* 28: 114–138.

YOUNG, A. G., T. BOYLE, AND T. BROWN. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* 11: 413–418.

YOUNG, A. G., A. H. D. BROWN, AND F. A. ZICH. 1999. Genetic structure of fragmented populations of the endangered daisy *Rutidosis leptorrhynchoides*. *Conservation Biology* 13: 256–265.

YOUNG, A. G., A. H. D. BROWN, B. G. MURRAY, P. H. THRALL, AND C. H. MILLER. 2000. Genetic erosion, restricted mating and reduced viability in fragmented populations of the endangered grassland herb *Rutidosis leptorrhynchoides*. *In* A. G. Young and G. M. Clarke [eds.], Genetics, demography and viability of fragmented populations, 335–359. Cambridge University Press, Cambridge, UK.

APPENDIX. Attributes of the 18 study sites for *Swertia perennis*: fen system number, location of fen system (Swiss canton in parentheses: SG = St. Gallen, SZ = Schwyz), habitat island type (Main = large; Near = small, barely isolated; Distant = small, isolated), name of fen, coordinates as in Swiss topographical maps (Swiss grid in meters[a]), altitude (in meters above sea level), distance of small island (Near/Distant) to Main island, island area, areal extent of population, population size (number of flowering adults), and surrounding habitat for the 18 study sites of *Swertia perennis*.

| No. | Fen system | Island type | Fen | Longitude[a] (m) | Latitude[a] (m) | Altitude (m a.s.l.) | Distance to Main island (m) | Island area (ha) | Areal extend (ha) | Population size | Surrounding habitat |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Grabs (SG | Main | Hirzenbäder | 747 650 | 227 300 | 1340 | | 5.73 | 3.00 | 118 500 | Meadows, woodland |
| | | Near | Herti | 747 550 | 227 025 | 1340 | 45 | 0.20 | 0.11 | 917 | Meadows, woodland |
| | | Distant | Maienberg | 748 300 | 226 575 | 1190 | 800 | 0.45 | 0.10 | 1287 | Meadows |
| 2 | Wildhaus (SG) | Main | Bilchenmoos | 748 100 | 230 400 | 1210 | | 6.51 | 6.51 | 65 110 | Meadows, woodland |
| | | Near | Sommerigweid | 748 300 | 230 525 | 1230 | 40 | 0.57 | 0.16 | 2772 | Woodland |
| | | Distant | Schönenboden | 746 950 | 230 300 | 1130 | 925 | 0.52 | 0.20 | 1562 | Meadows, agricultural land |
| 3 | Ebnat-Kappel, Hemberg (SG) | Main | Chellen, large | 730 700 | 237 350 | 1080 | | 4.28 | 4.28 | 32 100 | Woodland |
| | | Near | Chellen, small | 730 425 | 237 175 | 1070 | 106 | 0.54 | 0.33 | 6738 | Woodland, agricultural land |
| | | Distant[b] | Allmeindswald | 731 550 | 237 625 | 1070 | 750 | 0.20 | — | — | Woodland |
| 4 | Einsiedeln/Alpthal (SZ) | Main | Eigenrieter, large | 697 850 | 215 900 | 990 | | 7.47 | 0.60 | 2400 | Agricultural land |
| | | Near | Eigenrieter, small | 697 750 | 215 750 | 990 | 44 | 0.20 | 0.09 | 985 | Agricultural land |
| | | Distant | Etteren | 696 975 | 213 250 | 1020 | 1825 | 0.87 | 0.20 | 1348 | Meadows, agricultural land |
| 5 | Unteriberg, Studen (SZ) | Main | Chilenried, large | 705 600 | 214 750 | 895 | | 5.43 | 0.51 | 758 | Woodland, agricultural land |
| | | Near | Chilenried, small | 705 500 | 214 675 | 900 | 125 | 0.66 | 0.08 | 250 | Woodland, agricultural land |
| | | Distant | Ort | 706 475 | 213 900 | 910 | 1200 | 0.18 | 0.18 | 4446 | Agricultural land |
| 6 | Sattel (SZ) | Main | Zäll, large | 692 050 | 213 975 | 1130 | | 6.68 | 2.66 | 21 256 | Woodland, agricultural land |
| | | Near | Zäll, small | 691 875 | 213 875 | 1110 | 60 | 0.22 | 0.15 | 820 | Agricultural land |
| | | Distant | Gigersberg | 691 275 | 213 500 | 1010 | 800 | 0.57 | 0.002 | 7 | Agricultural land |

[a] As reference point we give Grabs Main island also in geographic coordinates: 9°23′E/47°11′N.
[b] There were no *S. perennis* on this island.