

Regional population dynamics define the local genetic structure in *Sorbus torminalis*

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Abstract

Recent changes in silvicultural practices in Central Europe have created forests with closed canopies, and tree species preferring open and sunny forests have declined in area and abundance. This led to increased isolation of populations of many rare insect-pollinated, fleshy-fruited species with a naturally scattered distribution. To gain insight into the regional population dynamics of such species, we investigated the consequences of spatial isolation, population size and density on the genetic structure of *Sorbus torminalis* and simultaneously considered the relationship between fecundity and habitat quality. Genotype data for biparentally (ISSRs) and maternally inherited (cpDNA PCR-RFLPs) molecular markers were generated for 26 Swiss populations of *S. torminalis*. We applied analyses of molecular variance (AMOVA) to both marker types and separated the relative contributions of pollen and seed dispersal to historical gene flow. AMOVA detected significant differentiation among populations ($\Phi_{ST\ ISSR} = 0.107$; $\Phi_{ST\ cpDNA} = 0.370$) in both marker types. The relative rate of pollen to seed gene flow was low ($r = 2.919$) and significantly different from equality. Isolation by distance was weak within Eastern and Western Switzerland, although populations were substantially differentiated. Within-population molecular variance was not explained by population size, whereas habitat quality (openness) positively influenced the percentage of fruiting trees and the degree of fruiting per tree, indicating that more open forests enhance sexual reproduction. Our findings of significant genetic differentiation in the absence of clear geographical structuring can be explained by the distinct ecology of *S. torminalis* and nondirectional colonization events in metapopulation-like dynamics.

Keywords: cpDNA-RFLP, genetic variation, insect-pollinated tree, ISSR, metapopulation, population density

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Introduction

The anthropogenic influence on forest habitats in Central Europe has significantly decreased during the last century, owing to strict forest legislations (Bürgi & Schuler 2003) and altered forestry management aiming to produce dense forests with closed canopies (Wohlgemuth *et al.* 2002; Bradshaw 2004). This led to a stabilization and even an increase in forest area and to the wide occurrence of seminatural stands with closed canopies (Bürgi & Schuler 2003). Ironically, this positive development also had

negative effects. As most forests have grown denser and darker, species preferring open woodlands encountered less favourable habitat conditions and became scarce (Wohlgemuth *et al.* 2002). These latter species had indirectly benefited from previous traditional silvicultural practices like coppicing, coppicing with standards or woodland pasturing (Bürgi & Schuler 2003), as dominant and competitive tree species were periodically suppressed. In other words, while for late successional species the habitat has been notably defragmented during the last century, the opposite happened to species favouring open or less-closed forests (Bradshaw 2004). Consequently, the habitat of these early successional species became less favourable, which potentially had a negative influence on their fecundity.

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Moreover, populations have become less abundant, and the remnant stands smaller and more isolated (Barengo *et al.* 2001). This process possibly induced demographic and genetic processes that negatively affected the persistence of several rare European tree species (Gibbs 2001; Bradshaw 2004).

A species sharing the described fate in Central Europe is the insect-pollinated, fleshy-fruited wild service tree *Sorbus torminalis* (L.) Crantz. It is a light-demanding pioneer tree that naturally colonizes slightly dry, shallow soils and steep slopes. It also occurs in disturbed forests and forest edges, but becomes suppressed by late-successional competitors and is never found in pure stands (Demesure *et al.* 2000). Hence, altered forest management leading to closed canopies can be expected to negatively affect *S. torminalis*' fecundity (Oddou-Muratorio *et al.* 2005). *S. torminalis* has recently been the subject of intensive genetic research. Contemporary pollen flow has been studied in two large and continuous populations, as well as in a spatially isolated stand (Oddou-Muratorio *et al.* 2003, 2005; Hoebee *et al.* WSL Birmensdorf, unpublished data). The studies revealed that external gene flow by pollen is about 30% in continuous populations and decreases to about 4% in populations separated by more than 1 km, suggesting that pollen flow is mainly local and reduced among scattered populations. Demesure *et al.* (2000) and Oddou-Muratorio *et al.* (2001) also investigated the genetic structure of *S. torminalis* at the large spatial scale throughout France using both nuclear and cpDNA molecular markers. They inferred a relatively high population differentiation along with weak geographical structure and a high proportion of gene flow by seed in relation to gene flow by pollen ($r = 2.21$) as compared with other tree species (Ennos 1994; Petit *et al.* 2005). These results suggest that the regional population dynamics, and therefore the genetic structure of *S. torminalis*, could be driven by generally weak gene flow, where pollen flow is only about twice as high as seed dispersal.

Changes in forest structure can affect the regional population dynamics of tree species depending on the quality of those changes. Therefore, this present study will investigate both the consequences of spatial isolation, population size and density on genetic variation as well as the influence of reduced habitat quality on fecundity in a light-demanding, early successional forest tree. Our study on *S. torminalis* has three objectives. First, we infer historical gene flow using neutral molecular markers with different modes of inheritance in order to separate the contributions of pollen flow and seed dispersal to genetic structure. We use inter simple sequence repeats (ISSRs) as biparentally inherited, dominant markers (Wolfe *et al.* 1998) and cpDNA polymerase chain reaction–restriction fragment length polymorphisms (PCR-RFLPs) as maternally inherited, haploid markers (Oddou-Muratorio *et al.* 2001) and apply the same statistical analyses to both marker types taking into

account different degrees of spatial isolation of populations. Second, we evaluate the general relationship of within population genetic variation of both marker types with current population census size. Third, we correlate approximate fitness-measurements (fruiting) with an index of habitat quality (openness).

Materials and methods

The species

Sorbus torminalis is a valuable timber species and has a widespread distribution from the Northern Maghreb through Central and Southern Europe to the Caspian Sea (Kutzelnigg 1995). In Northern Switzerland, *S. torminalis* mainly occurs along the Jura Mountains (Fig. 1), where its populations are scattered and spatially isolated from each other. Tree density within populations is often low and varies from only 0.5–30 individuals per hectare (Oddou-Muratorio *et al.* 2001).

In spring, the species produces hermaphroditic flowers, which are visited by generalist pollinators and are supposed to possess a relaxed self-incompatibility system (Oddou-Muratorio *et al.* 2003, 2005). Genetic paternity analyses from France and Switzerland showed that some trees partially self and sometimes do so at a substantial rate (up to 60%; Oddou-Muratorio *et al.* 2005; Hoebee *et al.* WSL Birmensdorf, unpublished data). In fall, the fleshy fruits are dispersed by birds such as thrushes, and mammals like foxes and martens (Rasmussen & Kollmann 2004). *S. torminalis* also shows clonal growth through root suckers, and clones usually expand over areas of 20–30 m in diameter (Hoebee *et al.* 2006).

Sample collection and field data

The sampling consisted of 15 large populations ($N \geq 100$) and 11 small populations ($N < 100$) of *S. torminalis* distributed throughout Northern Switzerland and separated by a mean geographical distance of 18 km (Fig. 1). As a consequence of the higher population density in Eastern Switzerland, the populations were assigned into the two groups East and West (18 populations and eight populations, respectively; Fig. 1). Population censuses and leaf tissue collections were carried out in 2000 and 2001 considering only trees with a diameter at breast height (d.b.h.) ≥ 6 cm. In order to avoid sampling from ramets of the same genet, plant material was only taken from trees separated by more than 40 m. When populations were smaller than 24 individuals, plant material from all individuals was collected, otherwise, 24 individuals were randomly chosen. Fresh plant material was transported to the laboratory and frozen at -20 °C. The total genetic data set consisted of 573 individuals from 26 populations (Table 1).

Table 1 Longitude and latitude according to Swiss grid coordinates, census population size and genetic sample size, molecular variance, mean habitat openness (for explanation see text), mean fruiting and percentage of fruiting trees per population, local population density within radii of 3 km and 6 km and mean Φ_{ST} to the nearest three surrounding populations (Φ_{ST3}) of 26 investigated Swiss *Sorbus torminalis* populations

| Population (abbreviation) | Longitude | Latitude | Census size | Genetic sample size* | Molecular variance* | Mean habitat openness | Mean fruiting | Fruiting trees (%) | 3 km | 6 km | Φ_{ST3} * |
|----------------------------------|-----------|----------|-------------|----------------------|---------------------|-----------------------|---------------|--------------------|------|------|----------------|
| Satigny (SAT) | 489.63 | 119.35 | 196 | 8/24 | 0.82/5.83 | 0.06 | 0.14 | 14 | 2 | 5 | 0.25/0.11 |
| La Foretaille (LFA) | 498.68 | 125.13 | 144 | 8/24 | 0.00/6.06 | 0.04 | 0.02 | 2 | 1 | 1 | 0.24/0.11 |
| Les Petit Lac (LPL) | 524.78 | 167.60 | c. 2000 | 8/24 | 0.43/6.67 | 0.28 | 0.18 | 16 | 6 | 11 | 0.23/0.11 |
| La Sarraz (LSZ) | 531.90 | 168.45 | 51 | 8/14 | 0.96/4.68 | 0.28 | 0.40 | 28 | 5 | 10 | 0.15/0.20 |
| Mont de Chamblon (MOC) | 534.95 | 180.20 | 203 | 8/24 | 0.25/5.00 | 0.18 | 0.32 | 30 | 0 | 0 | 0.34/0.13 |
| Prise Gaulaz (PRI) | 546.40 | 190.50 | 37 | 8/22 | 0.25/4.90 | 0.31 | 0.26 | 17 | 1 | 3 | 0.41/0.13 |
| Forêt de Bevaix (FOR) | 551.65 | 196.90 | 250 | 8/24 | 0.96/6.48 | 0.56 | 1.04 | 64 | 5 | 11 | 0.11/0.12 |
| La Forêt de l'Eter (LFE) | 567.13 | 210.78 | 243 | 8/24 | 0.00/5.78 | 0.24 | 0.34 | 30 | 8 | 18 | 0.37/0.08 |
| Les Montes (LMA) | 571.05 | 257.28 | 160 | 8/24 | 1.32/6.73 | 0.70 | 1.18 | 64 | 13 | 21 | 0.18/0.08 |
| Derrière la Vieille Eglise (DVA) | 594.55 | 249.43 | 68 | 8/24 | 0.68/6.13 | 0.64 | 0.41 | 34 | 6 | 12 | 0.08/0.06 |
| Chlekenberg (CHL) | 619.30 | 254.30 | 200 | 8/24 | 1.61/5.63 | 0.50 | 0.38 | 30 | 12 | 28 | 0.17/0.06 |
| Rebholden (REN) | 619.45 | 259.03 | 115 | 8/24 | 1.14/5.64 | 0.76 | 0.66 | 48 | 9 | 34 | 0.28/0.03 |
| Längstich (LGS) | 621.50 | 238.63 | 42 | 8/21 | 1.07/5.46 | 0.81 | 1.03 | 53 | 0 | 1 | 0.18/0.10 |
| Tannenboden (TAN) | 622.53 | 252.08 | 70 | 8/23 | 0.25/5.41 | 0.54 | 0.80 | 62 | 11 | 24 | 0.25/0.06 |
| Landschachen (LSD) | 623.73 | 256.30 | 181 | 8/24 | 0.54/6.21 | 0.64 | 1.18 | 56 | 10 | 36 | 0.22/0.04 |
| Schibliweg (SCH) | 637.23 | 242.55 | 141 | 8/23 | 0.00/6.23 | 0.58 | 0.64 | 48 | 6 | 11 | 0.19/0.10 |
| Zürihölzli (ZUR) | 653.60 | 258.88 | 132 | 8/21 | 0.00/5.65 | 0.50 | 0.32 | 30 | 10 | 37 | 0.43/0.08 |
| Altberg (ALT) | 673.55 | 254.15 | 84 | 8/23 | 0.79/6.98 | 0.24 | 0.24 | 16 | 3 | 6 | 0.37/0.06 |
| Reppischtal (REP) | 672.88 | 247.80 | 72 | 8/24 | 0.68/5.60 | 0.30 | 0.32 | 16 | 0 | 8 | 0.28/0.09 |
| Rosshaus (ROS) | 681.33 | 278.98 | 532 | 8/24 | 0.68/6.36 | 0.28 | 0.42 | 28 | 7 | 20 | 0.06/0.04 |
| Heiligfohrenhau (HEF) | 684.33 | 287.65 | 92 | 8/24 | 0.93/5.91 | 0.16 | 0.02 | 2 | 20 | 39 | -0.01/0.05 |
| Winzlerboden (WIN) | 688.00 | 274.00 | 16 | 8/11 | 0.43/6.36 | 0.13 | 0.25 | 25 | 9 | 18 | 0.04/0.05 |
| Längenberg (LGB) | 689.18 | 288.18 | 420 | 8/23 | 0.25/5.81 | 0.42 | 0.48 | 38 | 20 | 44 | 0.08/0.05 |
| Hochwacht (HOK) | 697.80 | 256.80 | 23 | 8/15 | 0.54/6.53 | 0.06 | 0.00 | 0 | 12 | 24 | -0.07/0.05 |
| Türni (TUR) | 698.70 | 277.46 | 31 | 8/16 | 0.54/5.44 | 0.55 | 1.16 | 71 | 15 | 25 | 0.10/0.06 |
| Bietenhart (BIT) | 715.30 | 268.93 | 250 | 8/24 | 0.25/7.48 | 0.56 | 1.04 | 64 | 15 | 26 | -0.01/0.06 |

*cpDNA PCR-RFLPs/ISSRs.

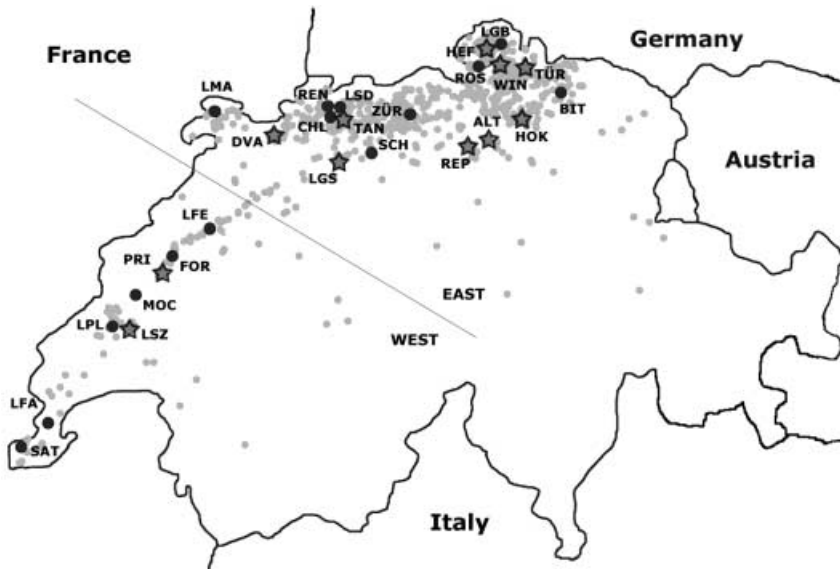


Fig. 1 Distribution of *Sorbus torminalis* populations in Switzerland (grey dots) according to Barenco *et al.* (2001). Black dots designate large ($N > 100$) and stars small study populations ($N < 100$). The grey line indicates the division into the groups Eastern and Western Switzerland. Population abbreviations follow Table 1.

Qualitative estimates of fecundity and habitat quality were recorded in autumn 2003. Again, only trees with a d.b.h. of ≥ 6 cm were considered. When populations comprised fewer than 50 individuals, all trees were surveyed, otherwise, 50 trees were randomly chosen. Fruiting was estimated by assigning trees to one of the following ranked fruiting classes: (0) 0%, (1) 1–25%, (2) 26–50%, (3) 51–75%, and (4) 76–100% of the tree crown covered with fruits. Habitat openness, as a measurement of habitat quality, was recorded by classifying the environment of individual trees as either (0) under canopy (1) open position within forest and (2) forest edge or remaining trees in forest clearings, an approach similar to Oddou-Muratorio *et al.* (2005).

DNA extraction

Fifty nanograms of frozen plant material were lyophilized, placed in liquid nitrogen for 10 min and disrupted using a Mixer Mill MM 300 (QIAGEN). DNA was isolated following the DNeasy 96 Plant Kit protocol (QIAGEN) with minor modifications: the amount of extraction buffer AP1 was increased to 600 μL , and DNA was eluted twice with 100 μL buffer AE. Extracted DNA was quantified against lambda DNA on 1% agarose gels in $1 \times$ TBE buffer, stained with ethidium bromide and visualized using a GDS 8000 documentation system (UVP).

ISSR analysis

The inter simple sequence repeat (ISSR) method utilizes a single primer consisting of a sequence of dinucleotide repeats (SSR, microsatellite) with a flag of one to three nucleotides at the 5' end to amplify the region in between adjacent priming sites (Wolfe *et al.* 1998). From the hundred primers of the UBC Primer set no. 9 (Biotechnology

Laboratory, University of British Columbia), 12 were randomly chosen to screen 12 individuals (randomly selected from different populations) for polymorphism. Five primers (no. 807, 834, 835, 848 and 857) were selected owing to consistent amplification, clear banding patterns and sufficient variation.

PCR amplifications for all the 573 individuals were performed in 20 μL reaction mixtures containing 2 ng of template DNA, $1 \times$ PCR buffer, 2 mM MgCl_2 , 0.1 mM of each dNTP, 0.2 μM of forward and reverse primer, 0.6 U of DNA *Taq* polymerase (Sigma-Aldrich) and 6.63 μL of double-distilled water. PCRs were carried out on a PTC-100 thermocycler (MJ-Research) with initial denaturation at 94 $^\circ\text{C}$ for 5 min, followed by 45 cycles of denaturation at 94 $^\circ\text{C}$ for 45 s, annealing at 51 $^\circ\text{C}$ or 54 $^\circ\text{C}$ for 45 s and extension at 72 $^\circ\text{C}$ for 1.5 min, ending with a final extension at 72 $^\circ\text{C}$ for 7 min. Annealing temperatures were 51 $^\circ\text{C}$ for the primers 834, 835 and 857 and 54 $^\circ\text{C}$ for the primers 807 and 848. PCR products were run on 2% agarose gels against 100 bp ladders in $1 \times$ TBE buffer. Gels were stained and photographed as described above.

cpDNA PCR-RFLP analysis

Twenty-three individuals from different populations were screened for polymorphism by using the whole set of cpDNA primers described in Demesure *et al.* (1995) in combination with eight restriction enzymes (*AluI*, *DdeI*, *HaeIII*, *HinfI*, *EcoRI*, *TaqI*, *MseI* and *SrfI*). This allowed the detection of length mutations in the following three primer/enzyme combinations: *trnS-trnFM/DdeI*, *trnD-trnT/MseI* and *trnC-trnD/MseI*. These combinations were then used to process a subset of eight randomly selected individuals per population (total $N = 208$). This number of samples is sufficient to estimate the genetic population structure of

uniparentally inherited organelle genomes (Pons & Petit 1995). PCR amplifications were performed in 15 µL reaction mixtures containing 2 ng of template DNA, 1 × PCR buffer, 1.6 mM MgCl₂, 0.1 mM of each dNTP, 0.2 µM of forward and reverse primer, 0.6 µg of BSA, 0.75 U of DNA *Taq* polymerase (Sigma-Aldrich) and 6.63 µL of double-distilled water. PCRs were carried out on a PTC-100 thermocycler (MJ Research) with initial denaturation at 94 °C for 4 min, followed by 30 cycles of denaturing at 94 °C for 45 s, annealing at 54.5 °C, 58 °C or 62 °C for 45 s and extension at 72 °C for 2 min (or 3 min for *trnC-trnD*), ending with a final extension at 72 °C for 10 min. Annealing temperatures were 54.5 °C for the primer pair *trnD-trnT*, 58 °C for *trnC-trnD* and 62 °C for *trnS-trnM*. Restrictions were completed at 37 °C overnight in a total volume of 10 µL containing 5 µL of PCR product, 1 × restriction buffer, 2 U of restriction enzyme (Fermentas), 1 µg BSA and 3.7 µL of double-distilled water. Restriction products were separated and visualized as described above.

Data analysis

The dominant ISSR markers were treated as multilocus haplotypes and analysed in the same way as the cpDNA PCR-RFLP haplotypes. Banding patterns were scored by eye and coded in a binary form for band presence (1) or absence (0). AMOVA-PREP (Miller 1998) was used to create input files.

Three approaches were used to estimate the effects of spatial isolation on the genetic structure in *S. torminalis*. First, population differentiation was evaluated with analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) in ARLEQUIN version 2.000 (Schneider *et al.* 2000) using 1000 permutations for the entire data set with the two groups East and West (Fig. 1). In order not to change the total sum of squares, population differentiation within these two groups was obtained by calculating the mean of all pairwise Φ_{ST} values among populations within each group separately. Second, one-dimensional isolation-by-distance analyses were performed with Mantel tests between linearized estimates of genetic differentiation of pairs of populations ($\Phi_{ST}/1 - \Phi_{ST}$) and corresponding geographical distances. Mantel tests were conducted with 1000 permutations in ARLEQUIN version 2.000 over all populations within Switzerland and for Eastern and Western Switzerland separately. Third, the regional relationship between population density and population differentiation was tested. For this purpose, the regional population density was estimated twice; first as the number of surrounding populations within a radius of 3 km from the centre of each target population and likewise for a radius of 6 km. These spatial dimensions reflect the lower and upper boundaries of geographical isolation in Swiss *S. torminalis* populations (Barengo *et al.* 2001). The number of surrounding populations was counted from the Swiss distribution data of *S. torminalis*

derived from a complete population survey in Northern Switzerland based on the knowledge of local foresters (Barengo *et al.* 2001). To measure regional population differentiation, we calculated the mean pairwise Φ_{ST} value of each target population with its geographically closest three genotyped populations (Φ_{ST3} ; Table 1). Population densities at 3 km and 6 km as well as Φ_{ST3} -values were checked for normal distribution using Kolmogorov–Smirnov tests and correlated by Pearson correlation coefficients with Bonferroni adjustment.

To estimate the overall relative rates of seed and pollen flow among populations, the ratio of pollen (m_p) to seed migration (m_s) was calculated with the equation:

$$m_p/m_s = [(1/F_{STb} - 1) - 2(1/F_{STm} - 1)] / (1/F_{STm} - 1),$$

where F_{STb} is the biparentally and F_{STm} the maternally inherited differentiation among populations (Ennos 1994). The $\Phi_{ST\text{ISSR}}$ -value from AMOVA was used as the biparental and the $\Phi_{ST\text{cpDNA}}$ value as the maternal estimate of differentiation (Oddou-Muratorio *et al.* 2001). Assuming that pollen and seed migration rates are identical and mutation rates are similar, organelle haplotypes should exhibit up to three times the fixation among populations as nuclear alleles (Hamilton & Miller 2002). Under this null hypothesis, the following relationship between the F_{ST} values for organelle and nuclear genomes is expected for equilibrium populations of outcrossing hermaphrodite plants with strictly maternal inheritance of organelles:

$$F_{STc} = a_1 F_{STn} / [a_2 + (a_1 - a_2) F_{STn}],$$

where $a_1 = 6$ and $a_2 = 2$ (Hamilton & Miller 2002). This equation was used to calculate the theoretically expected value of nuclear $\Phi_{ST\text{ISSR}}$ given the observed chloroplast $\Phi_{ST\text{cpDNA}}$, and the expected value was compared with the observed $\Phi_{ST\text{ISSR}}$ -value. To obtain a standard error for the observed nuclear Φ_{ST} , 5000 bootstrap samples (random sampling of individuals within populations) were generated in R version 2.0.1 (R Development Core Team 2004). The bootstrap standard error was used to calculate a 95% confidence interval for the observed nuclear Φ_{ST} .

To analyse the general relationship of within population genetic variation of both marker types with current population census size, Spearman rank correlation coefficients with Bonferroni adjustment were used. The relative molecular variance of both genetic markers was calculated as the AMOVA sums of squares of each population (WINAMOVA version 1.55) divided by $n - 1$ (Fischer & Matthies 1998), where n is the number of analysed individuals per population.

Similarly, to evaluate the general relationship of habitat quality, fecundity and population size in *S. torminalis*, the percentage of fruiting trees and the means of fruiting and habitat openness classes per population were calculated

(Table 1). These values were then correlated with each other and with census population size using Spearman rank correlation coefficients with Bonferroni adjustments. All standard statistical tests were performed using SPSS version 11.0.1 (SPSS 2001).

Results

Both marker types showed high levels of genetic diversity. The five ISSR primers used to process 573 individuals generated 54 polymorphic ISSR fragments, resulting in 570 different multilocus genotypes. The number of ISSR fragments amplified per primer varied from seven to 13 across populations, with an average of 10.8. The three cpDNA primer/enzyme combinations used to process 208 individuals identified nine length mutations, which resulted in 10 different haplotypes (Fig. 2). Six of them

were shared by two or more populations, while four were private. Haplotype 3 was the most frequent (56%) and widely distributed (present in 23 of 26 populations), followed by haplotype 1 (15%; 13 populations) and haplotype 4 (15%; 10 populations). The frequencies of the remaining haplotypes were all below 0.05. Only four populations were fixed for a single haplotype (Fig. 2).

AMOVA revealed significant population differentiation and were consistent for both marker types. ISSR markers generally exhibited lower levels of differentiation than cpDNA PCR-RFLP markers, and there was a significant differentiation among the two groups East and West as well as among populations within these groups for both marker types (Table 2). The population differentiation was larger within than among groups and stronger in Western Switzerland than Eastern Switzerland (Table 2). There was no significant overall correlation between population

Table 2 Results of analyses of molecular variance (AMOVAs) of *Sorbus torminalis* populations in entire Switzerland and the mean of all pairwise Φ_{ST} values among populations in the two groups Western and Eastern Switzerland for both ISSR and cpDNA PCR-RFLP markers

| Source of variation | ISSR | | | cpDNA | | |
|---|------|----------|---------------------|-------|---------|---------------------|
| | d.f. | MS | % variation | d.f. | MS | % variation |
| Switzerland | | | | | | |
| Among groups (Φ_{CT}) | 1 | 58.327 | 2.40*** | 1 | 11.332 | 10.55** |
| Among populations within groups (Φ_{SC}) | 24 | 433.967 | 8.25*** | 24 | 61.726 | 26.42*** |
| Within populations (Φ_{IS}) | 547 | 3264.020 | 89.35*** | 182 | 107.500 | 63.03*** |
| Among populations (Φ_{ST}) | | | 10.65*** | | | 36.97*** |
| Western Switzerland | | | | | | |
| Mean pairwise Φ_{ST} among populations | | | 12.58 ^{NA} | | | 37.88 ^{NA} |
| Eastern Switzerland | | | | | | |
| Mean pairwise Φ_{ST} among populations | | | 6.11 ^{NA} | | | 15.64 ^{NA} |

***, $P \leq 0.001$; **, $P \leq 0.01$; ^{NA}, not assessed.

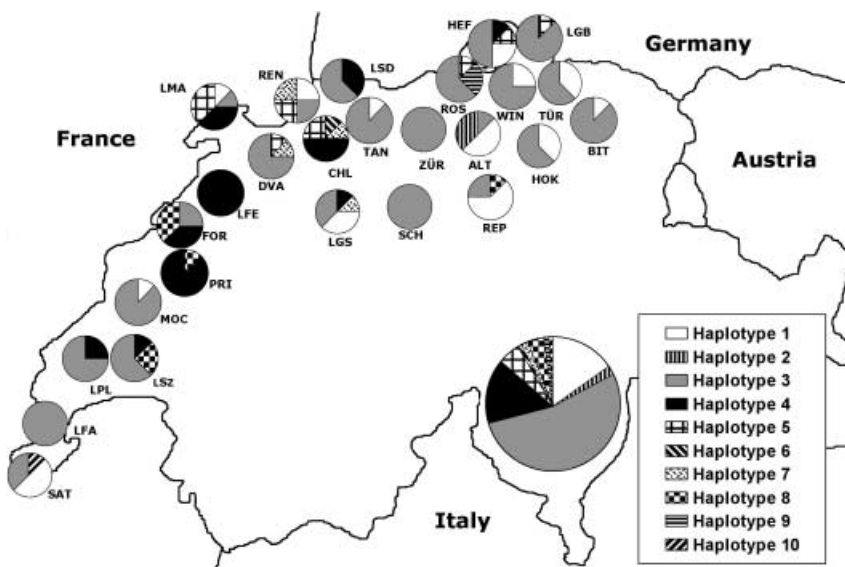


Fig. 2 Geographical distribution of 10 cpDNA haplotypes identified in Swiss *Sorbus torminalis* populations. Shading of the small pie charts corresponds to haplotype proportions within populations, whereas the shading of the large pie chart gives the relative frequencies of haplotypes across all populations. Population abbreviations follow Table 1.

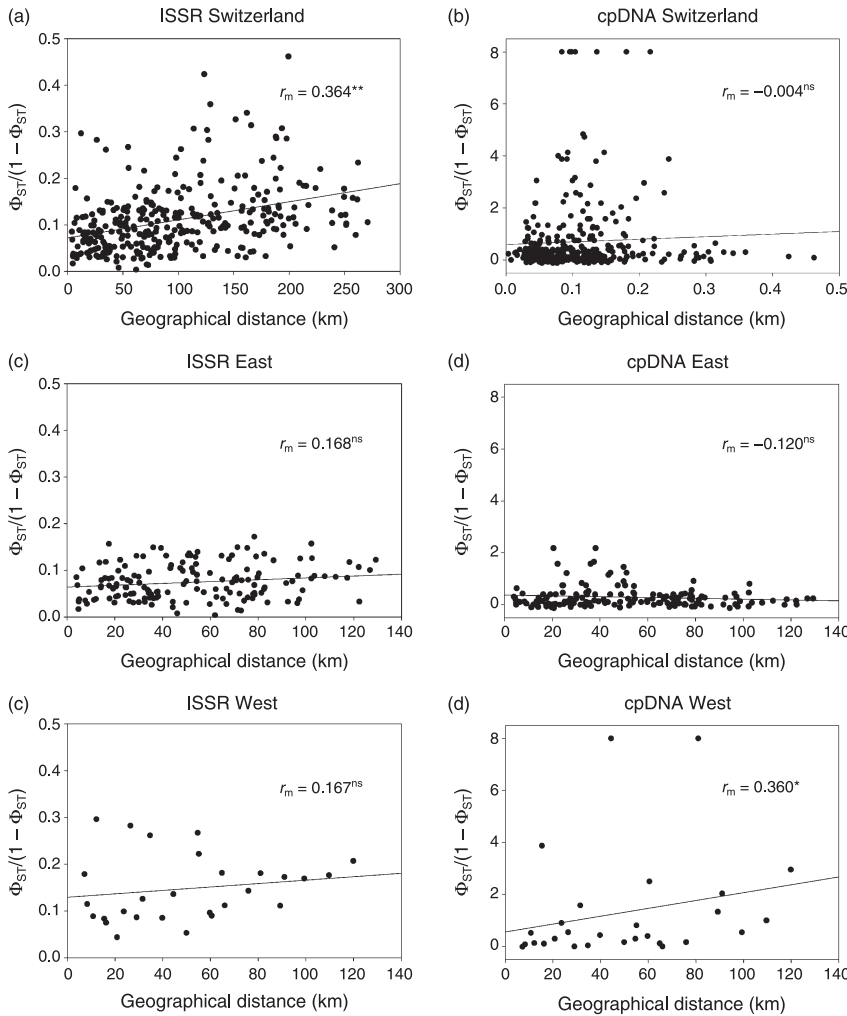


Fig. 3 Isolation by distance (Mantel tests) of *Sorbus torminalis* populations across Switzerland (a, b) and within Eastern (c, d) and Western Switzerland (e, f) for both ISSR (a, c, e) and cpDNA PCR-RFLP (b, d, f) markers. **, $P = 0.01$; *, $P = 0.05$; ns, not significant.

molecular variance and census population size in both marker types ($r_{s\text{ISSR}} = -0.106, P = 0.606$; $r_{s\text{cpDNA}} = 0.250, P = 0.905$).

Isolation by distance was apparent for ISSR markers across Switzerland ($r_m = 0.364, P \leq 0.01$) and for cpDNA PCR-RFLP markers in Western Switzerland ($r_m = 0.360, P \leq 0.05$; Fig. 3). In contrast, we found a significant negative general correlation between population density at distances of 3 km and 6 km and Φ_{ST3} for ISSR markers (Table 3). For cpDNA PCR-RFLP markers, this correlation was only significant for population densities at 3 km but not at 6 km (Table 3). This suggested that population differentiation decreases when *S. torminalis* populations are locally abundant, but increases when there are only few neighbouring populations. (Note that there was no sampling artefact, since the geographical distances to the closest three genotyped populations were not correlated with population densities at 3 km nor 6 km; Spearman rank correlation coefficient $r_s = -0.194$ with $P = 0.342$ and $r_s = -0.022$ with $P = 0.917$, respectively.)

The observed among population differentiation values from AMOVA for the maternally inherited markers and for

Table 3 Pairwise Pearson correlation coefficients calculated for the mean Φ_{ST} value to the nearest three genotyped populations (Φ_{ST3}) and population densities within radii of 3 km and 6 km around a target population in *Sorbus torminalis* from Switzerland

| | 3 km | 6 km |
|--------------------------|-----------|----------------------|
| $\Phi_{ST3\text{ISSR}}$ | -0.578** | -0.656*** |
| $\Phi_{ST3\text{cpDNA}}$ | -0.545*** | -0.317 ^{NS} |

***, $P \leq 0.001$; **, $P \leq 0.01$; ^{NS}, not significant.

the biparentally inherited markers (Table 2) resulted in a pollen to seed flow ratio of $r = 2.919$. The theoretically expected differentiation value for the biparentally ISSR markers calculated under the null hypothesis of equal pollen and seed migration rates was 0.164. As the bootstrap 95% confidence interval for the observed $\Phi_{ST\text{ISSR}}$ value was $0.094 \leq 0.107 \leq 0.120$, the expected value was significantly different from the observed. This pointed to a slightly, but

Table 4 Pairwise Spearman rank correlation coefficients calculated for the percentage of fruiting trees, mean fruiting, mean habitat openness (for explanations see text) and census population size of *Sorbus torminalis* populations from Switzerland

| Variables | Percentage of fruiting trees | Habitat openness | Census size |
|------------------------------|------------------------------|------------------|---------------------|
| Mean fruiting | 0.956*** | 0.859*** | 0.148 ^{NS} |
| Percentage of fruiting trees | | 0.833*** | 0.136 ^{NS} |
| Mean habitat openness | | | 0.011 ^{NS} |

***, $P \leq 0.001$; ^{NS}, not significant.

significantly higher level of historical pollen than seed migration among populations.

Census population size did neither correlate with mean habitat openness and mean fruiting nor with the percentage of fruiting trees per population, whereas the latter three variables were all highly correlated with each other (Table 4). These results show that larger populations do not occupy better habitat patches and that more open and sunny forests harbour *S. torminalis* trees that fruit more abundantly and more strongly.

Discussion

At first sight, our study seemed to reveal contradicting genetic results, as we found strong population differentiation but no clear indication of isolation by distance. Hence, we first discuss genetic differentiation and diversity and then explain how the apparent discrepancies can be attributed to the distinct population dynamics and ecology of *Sorbus torminalis*, with different processes acting at different spatial scales (Garcia *et al.* 2005).

Population differentiation

The two marker types used in this study detected unequal levels of neutral genetic differentiation in populations of *S. torminalis*. This is primarily due to the lower effective population size of the haploid chloroplast vs. the nuclear genome, enforcing the effects of genetic drift, and of asymmetrical migration of biparentally and maternally inherited genes in plants (Ennos 1994; Hamilton & Miller 2002). Our values of overall differentiation ($\Phi_{ST\ ISSR} = 0.107$ and $\Phi_{ST\ cpDNA} = 0.370$) were in good accordance with allozyme and cpDNA PCR-RFLP data obtained from *S. torminalis* populations in France ($G_{ST} = 0.11$ and 0.34 , respectively; Oddou-Muratorio *et al.* 2001), which, accordingly, led to similar over all proportions of historical gene flow by pollen and seed ($r = 2.92$ in this study; $r = 2.21$ in Oddou-Muratorio *et al.* 2001). However, when comparing

the Φ_{ST} -values of cpDNA and ISSR markers in Table 1, it becomes evident that the relative contributions of gene flow by pollen and of gene flow by seed show different regional asymmetries in *S. torminalis*. Under the assumption of historical equilibrium populations, *S. torminalis* is thus generally characterized by a relatively high ratio of gene flow by seed as compared to gene flow by pollen. This value, however, does not say anything about the effective amount of historical gene flow by pollen or seed, as both could be either high or low. Our value of overall $\Phi_{ST\ cpDNA}$ was also similar to corresponding values obtained for other woody Rosaceae (e.g. *Sorbus aucuparia*, $G_{ST} = 0.29$, Raspé *et al.* 2000; *Prunus spinosa*, $G_{ST} = 0.33$, Mohanty *et al.* 2002). Given that all these genetic surveys were performed at different geographical scales, the rather similar levels of population differentiation in cpDNA and/or nuclear DNA could be a general feature of sparsely distributed, insect-pollinated and fleshy fruited woody Rosaceae in Europe, and potentially reflect their distinct population dynamics (see below).

Sorbus torminalis showed significant genetic differentiation among Swiss populations for both marker types studied. Furthermore, populations in Western Switzerland exhibited stronger population differentiation than those from Eastern Switzerland. The total genetic diversity of populations in a fragmented landscape must not necessarily be reduced, but becomes patchier and more structured (Hamrick 2004). The more pronounced differentiation of the populations in Western Switzerland could therefore have resulted from higher spatial isolation due to lower population density in this area as compared with Eastern Switzerland. This interpretation is also supported by the negative correlations observed between population densities at 3 km and 6 km with mean pairwise Φ_{ST} -values. All these findings indicate that gene flow among Swiss populations has been relatively low, especially in Western Switzerland. Nevertheless, significant isolation by distance for ISSR markers was only apparent across Switzerland, but not so in Western and Eastern Switzerland. Moreover, isolation by distance for cpDNA PCR-RFLP markers was only detected in Western Switzerland (with a lower density of *S. torminalis* populations), but not in Eastern Switzerland or across Switzerland. As isolation-by-distance estimates are highly dependent on the geographical area investigated, the deviating results may simply be caused by analysing different spatial scales (Slatkin 1993). However, the lack of a consistent isolation-by-distance pattern for both marker types suggests that local populations of *S. torminalis* did not tend to be colonized from only nearby populations.

Providing that populations remain stable for longer time periods and that gene flow is more likely to occur between neighbouring populations, equilibrium between gene flow and drift will result in isolation by distance (Slatkin 1993; Hutchison & Templeton 1999). A lack of such a regional

equilibrium, as observed in this study, could point to metapopulation-like dynamics, where colonists originate from several geographically and genetically unrelated source populations, which in turn results in a patchy genetic structure. In the case of *S. torminalis*, colonization happens by seed dispersal via mainly birds or occasionally mammals (Oddou-Muratorio *et al.* 2001). After establishment of a new population, subsequent local gene flow by pollen through generalist pollinators such as bees (Oddou-Muratorio *et al.* 2003) seems to be generally low. Both processes eventually result in a genetic population differentiation pattern that is not regionally structured. *S. torminalis* contrasts with other tree species in its high proportion of gene flow through seed (Ennos 1994; Petit *et al.* 2005), which is easily understood given that fruits are mainly dispersed by birds. High proportions of long-distance gene flow by seed and its mechanisms have also been described for *S. torminalis* by Oddou-Muratorio *et al.* (2001) and for other woody Rosaceae by Jordano & Godoy (2000) or Bacles *et al.* (2004).

Genetic variation and habitat quality

Neutral genetic variation within *S. torminalis* populations was generally not related with population census size, even though it is theoretically expected to increase with population size (Ellstrand & Elam 1993). However, similar results have been described for other plants in fragmented habitats (Landerogott *et al.* 2001; Vergeer *et al.* 2003; Hamrick 2004). As an explanation, Young *et al.* (1996) stated that habitat fragmentation does not necessarily result in reduced genetic variation, especially when the time elapsed since fragmentation has been too short to leave detectable footprints in genetic diversity or differentiation. This scenario is very likely in the rather long-lived *S. torminalis* because the changes in silvicultural practices date back to not even one to three generations ago. Furthermore, *S. torminalis*' capacity of clonal reproduction enables the preservation of genetic variation within populations even under adverse ecological conditions (Hoebee *et al.* 2006). Hence, the genetic variation and structure observed in this study probably still reflects historical variation, although the altered forestry management could well have initiated a process changing their levels. In fact, the changes in silvicultural practices seem to influence the reproduction of *S. torminalis*, as we found a clear positive relationship between habitat openness, fruiting and the percentage of fruiting trees per population. The fecundity of a population thus largely depends on favourable habitat conditions and not on population size (Table 4). Our results, in accordance with those of Oddou-Muratorio *et al.* (2005), thus show that with ongoing succession, which leads to denser and darker forests, the fecundity of *S. torminalis* decreases substantially.

Regional population dynamics

The results of the present study are in line with the findings of Oddou-Muratorio *et al.* (2001) and support the conclusion that new populations of *S. torminalis* are founded by a random sample of seeds originating from populations encompassing a broad geographical area (low pollen-to-seed dispersal ratio). This results in nonspatially structured population differentiation (no isolation by distance but significant population differentiation within regions). Established populations then experience higher levels of local gene flow only when adjacent populations occur at higher densities (negative correlation between Φ_{ST3} and population density at 3 km and 6 km). Hence, we argue that it is mainly the gene flow by seed during the pioneer phase of a habitat patch that determines the genetic composition of local or newly founded *S. torminalis* populations. With ongoing succession and canopy closure by dominant trees, fewer *S. torminalis* trees within populations flower and fruit (positive correlation of habitat openness with percentage of fruiting trees and fruiting). Consequently, local populations of *S. torminalis* in later successional stages contribute to a lesser extent to the regional migrant gene pool (Slatkin 1977), but are still able to persist for extended time periods due to their capacity of clonal growth.

The described pattern of population colonization, persistence and extinction, where regional processes define local ones, corresponds to metapopulation-like dynamics as already proposed by Demasure *et al.* (2000) and Oddou-Muratorio *et al.* (2001). In forest trees, however, metapopulation-like dynamics should be taken in a relaxed sense, as they occur at time scales well over 100 years – a situation that makes the provision of empirical data on population extinction and colonization per generation a challenging task (Baguette 2004; Holderegger *et al.* in press). In Switzerland, former traditional silvicultural practices allowed the persistence of *S. torminalis* at a given site by either periodically creating suitable habitat patches for the species' recruitment and growth or by suppressing otherwise superior competitive tree species (Barengo *et al.* 2001; Bürgi & Schuler 2003). The recent changes in traditional forestry practices have led to a substantial decline of populations of *S. torminalis* and, possibly, to the disruption of its distinct metapopulation-like dynamics. The same might be true for several other, nowadays rare insect-pollinated, fleshy-fruited tree species of Central Europe, such as *S. domestica*, *Malus silvestris* and *Pyrus pyraeaster*. To conserve such rare species, solid knowledge of their regional population dynamics is needed.

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References

- Bacles CFE, Lowe AJ, Ennos RA (2004) Genetic effects of chronic habitat fragmentation on tree species: the case of *Sorbus aucuparia* in a deforested Scottish landscape. *Molecular Ecology*, **13**, 573–584.
- Baguette M (2004) The classical metapopulation theory and the real, natural world: a critical appraisal. *Basic and Applied Ecology*, **5**, 213–224.
- Barengo N, Rudow A, Schwab P (2001) *Förderung Seltener Baumarten (Translated: Promotion of Rare Trees)*. BUWAL, Bern.
- Bradshaw RHW (2004) Past anthropogenic influence on European forests and some possible genetic consequences. *Forest Ecology and Management*, **197**, 203–212.
- Bürgi M, Schuler A (2003) Driving forces of forest management – an analysis of regeneration practices in the forests of the Swiss Central Plateau during the 19th and 20th century. *Forest Ecology and Management*, **176**, 173–183.
- Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, **4**, 129–131.
- Demesure B, Le Guerroué B, Lucchi G, Prat D, Petit RJ (2000) Genetic variability of a scattered temperate forest tree: *Sorbus torminalis* L. (Crantz). *Annals of Forest Science*, **57**, 63–71.
- Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics*, **24**, 217–242.
- Ennos RA (1994) Estimating the relative rates of pollen and seed migration among plant populations. *Heredity*, **72**, 250–259.
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
- Fischer M, Matthies D (1998) RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). *American Journal of Botany*, **85**, 811–819.
- García D, Miller JR, Martínez I (2005) Spatial concordance between seed rain and seedling establishment in bird-dispersed trees: does scale matter? *Journal of Ecology*, **93**, 693–704.
- Gibbs JP (2001) Demography versus habitat fragmentation as determinants of genetic variation in wild populations. *Biological Conservation*, **100**, 15–20.
- Hamilton MB, Miller JR (2002) Comparing relative rates of pollen and seed gene flow in the island model using nuclear and organelle measures of population structure. *Genetics*, **162**, 1897–1909.
- Hamrick JL (2004) Response of forest trees to global environmental changes. *Forest Ecology and Management*, **197**, 323–335.
- Hoebee SE, Menn C, Rotach P, Holderegger H, Finkeldey R (2006) Spatial genetic structure of *Sorbus torminalis*: the extent of clonal reproduction in natural stands of a rare tree species with scattered distribution. *Forest Ecology and Management*, **226**, 1–8.
- Holderegger H, Gugerli F, Scheidegger C, Taberlet P (in press) Integrating population genetics with landscape ecology to infer spatio-temporal processes. In: *A Changing World – Challenges for Landscape Research* (eds Kienast F, Gosh R, Wildi O). Kluwer, Dordrecht, The Netherlands.
- Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.
- Jordano P, Godoy JA (2000) RAPD variation and population genetic structure in *Prunus mahaleb* (Rosaceae), an animal-dispersed tree. *Molecular Ecology*, **9**, 1293–1305.
- Kutzelnigg H (1995) *Sorbus torminalis*. In: *Gustav Hegi. Illustrierte Flora Von Mitteleuropa IV* (eds Conert HJ, Jäger EJ, Kadereit JW et al.), pp. 343–349. Blackwell, Berlin.
- Landergott U, Holderegger R, Kozłowski G, Schneller JJ (2001) Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata*. *Heredity*, **87**, 344–355.
- Miller MP (1998) *AMOVA-PREP 1.01. A Program for the Preparation of amova Input Files from Dominant Marker Raw Data*. Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona.
- Mohanty A, Martín JP, Aguinalde I (2002) Population genetic analysis of European *Prunus spinosa* (Rosaceae) using chloroplast DNA markers. *American Journal of Botany*, **89**, 1223–1228.
- Oddou-Muratorio S, Houot ML, Demesure-Musch B, Austerlitz F (2003) Pollen flow in the wild service tree, *Sorbus torminalis* (L.) Crantz. I. Evaluating the paternity analysis procedure in continuous populations. *Molecular Ecology*, **12**, 3427–3439.
- Oddou-Muratorio S, Klein EK, Austerlitz F (2005) Pollen flow in the wildservice tree, *Sorbus torminalis* (L.) Crantz. II. Pollen dispersal and heterogeneity in mating success inferred from parent-offspring analyses. *Molecular Ecology*, **14**, 4441–4452.
- Oddou-Muratorio S, Petit RJ, Le Guerroué B, Guesnet D, Demesure B (2001) Pollen- versus seed-mediated gene flow in a scattered forest tree species. *Evolution*, **55**, 1123–1135.
- Petit RJ, Duminil J, Fineschi S et al. (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology*, **14**, 689–701.
- Pons O, Petit RJ (1995) Estimation, variance and optimal sampling of gene diversity. I. Haploid locus. *Theoretical and Applied Genetics*, **90**, 462–470.
- R Development Core Team (2004) *ROYAL: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Rasmussen KK, Kollmann J (2004) Poor sexual reproduction on the distribution limit of the rare tree *Sorbus torminalis*. *Acta Oecologica*, **25**, 211–218.
- Raspé O, Saumitou-Laprade P, Cuguen J, Jacquemart AL (2000) Chloroplast DNA haplotype variation and population differentiation in *Sorbus aucuparia* L. (Rosaceae: Maloideae). *Molecular Ecology*, **9**, 1113–1122.
- Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN, Version 2.000: a Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva.
- Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253–262.
- Slatkin M (1993) Isolation by distance in equilibrium and nonequilibrium populations. *Evolution*, **47**, 264–279.
- SPSS (2001) *SPSS BASE 11.0.1 for Windows User's Guide*. SPSS, Chicago.
- Vergeer P, Rengelink R, Copal A, Ouborg NJ (2003) The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. *Journal of Ecology*, **91**, 18–26.
- Wohlgenuth T, Bürgi M, Scheidegger C, Schütz M (2002) Dominance reduction of species through disturbance – a proposed

- management principle for Central European forests. *Forest Ecology and Management*, **166**, 1–15.
- Wolfe AD, Xiang QY, Kephart SR (1998) Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. *Molecular Ecology*, **7**, 1107–1125.
- Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution*, **11**, 413–418.

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