

Contrasting effects of grazing and hay cutting on the spatial and genetic population structure of *Veratrum album*, an unpalatable, long-lived, clonal plant species

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Summary

1 Vegetation change induced by large herbivores is driven by the effects of grazers on populations of individual plant species. Short-term experimental or demographic studies may be insufficient when investigating the population responses of long-lived clonal plant species.

2 We therefore examined the effects of grazing on such a plant (*Veratrum album*) by comparing the spatial and genetic structure of populations in grasslands subject to long-term grazing or mowing for hay.

3 *V. album* is a locally dominant species that is avoided by large herbivores due to its toxicity. RAPD-phenotypes of a subsample of c. 50 shoots, and co-ordinates and dry weight of all shoots, were determined in a 5 × 10 m plot in each of four meadow and four pasture populations.

4 The breeding system of the genus *Veratrum* was previously unknown but our experimental finding that cross-pollinated but not self-pollinated or unpollinated flowers produced as many seeds as freely pollinated flowers suggested that *V. album* is a predominantly cross-pollinating species.

5 Both the spatial and genetic population structure differed markedly between the two grassland types. Clonal expansion of established plants in pastures led to populations consisting of larger shoots that were significantly more aggregated at a small spatial scale. Populations also had a higher proportion of flowering shoots, less seedling recruitment and a lower genotypic diversity in pastures than hay meadows.

6 The differences in population structure appear to be due to hay meadow populations reproducing primarily by seeds, whereas clonal reproduction accounts for half of the population growth in pastures. We suggest that, as livestock selectively avoids *V. album* shoots, grazing indirectly promotes plant growth, which results in an enhanced vegetative reproduction as well as a higher seed production. Experimental studies are, however, needed to determine why and how grazing adversely affects seedling recruitment.

7 Detailed information on population level responses of unpalatable dominant plant species, such as provided by the present study, may help us understand and predict vegetation change in response to changing levels of herbivory.

Key-words: breeding system, genotypic diversity, herbivores, mode of reproduction, RAPD-phenotype, spatial autocorrelation

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Introduction

Large herbivores have profound effects on plant community structure, composition and dynamics

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(McNaughton *et al.* 1997; Augustine & McNaughton 1998; Olff & Ritchie 1998). These effects may be caused indirectly by trampling, by the removal and subsequent patchy redistribution of nutrients, and by changing the chemical composition of the nutrients that are returned to the vegetation (Edwards & Hollis 1982; Bakker *et al.* 1983; Bakker 1987; Frank & McNaughton 1992; Steinauer & Collins 1995; Frank & Evans 1997). Ultimately, vegetation change is driven by effects on

populations of individual species, with some species expanding while others decline in response to herbivory (Crawley 1988). Although in theory the properties of the component species could enable predictions of vegetation change, in reality a large number of species are involved and, more importantly, their large differences in chemical composition, architecture, life history traits, competitive ability and grazing tolerance, make generalizations about the effects of herbivores impossible (Weiner 1993). Nevertheless, insight into the dynamics of plant populations as well as plant communities may be gained by studying effects of grazers on species that dominate the vegetation in the presence of but not in the absence of herbivores, or vice versa (e.g. Allen *et al.* 1984; Brown & Stuth 1993; Anderson & Briske 1995; Augustine & Frelich 1998).

There are few detailed studies of the effects of grazers on population structure (e.g. see Mulder 1999 for an overview of subarctic and arctic ecosystems) and most studies of these focus on monitoring the fate of individual plants at varying levels of herbivory (Watkinson 1986). Such studies face two major challenges. First, most species are able to propagate vegetatively as well as sexually (Bullock *et al.* 1994; de Kroon & Bobbink 1997; Klimeš *et al.* 1997) and herbivores often have different effects on propagation via ramets and via seeds (Fernández *et al.* 1992; Kerley *et al.* 1993; Bullock *et al.* 1994). Secondly, many species are very long-lived (e.g. 2000 years for clones of the sedge *Carex curvula* in an alpine grassland, Steinger *et al.* 1996) making extrapolation from findings of short-term experimental or demographic studies to natural populations unreliable (Amat 1995; Mulder 1999). Observed patterns in distributions of long-lived plants may more accurately reflect past conditions than those imposed by experimental treatments. Schlöpfer & Fischer (1998), who found no effects of 16 years of mowing or burning on the population structure of the clonal grass *Brachypodium pinnatum*, concluded that this species took longer than the experimental time to respond. Furthermore, demographic studies may underestimate the relative importance of rarely occurring stage transitions; for example, seedling establishment, although infrequently, may still contribute significantly to the population structure of a species if it is long-lived (Watkinson & Powell 1993).

Alternatively, the effects of herbivory on the spatial and genetic population structure can be used to infer the most likely processes involved. Recently developed molecular techniques make it possible to determine the size and distribution of individual clones (e.g., Steinger *et al.* 1996; Ayres & Ryan 1999; Pellegrin & Hauber 1999; Burke *et al.* 2000) and thus separate the contributions of sexual and vegetative reproduction to population growth. Furthermore, the efficiency of some of these techniques allows many populations to be sampled and therefore a sound assessment of treatment effects.

Veratrum album L. (Liliales, Melianthaceae) is a herbaceous species of (sub)alpine grasslands that has

an estimated life span of several centuries and propagates both through seeds and clonally. All plant parts contain high concentrations of alkaloids and are unpalatable to cattle (Binns *et al.* 1972; Mulligan & Munro 1987) and the species can therefore achieve local dominance following grazing. Its occurrence in both traditional hay meadows and pastures allows us to examine the effects of long-term grazing on the population structure by comparing fields where vegetation biomass has been removed selectively by grazers with that in mown grasslands, where vegetation has been removed irrespective of the palatability of plant species but conditions are otherwise similar. In the study area (the Swiss Alps below the tree line), grasslands represent an arrested stage of succession towards forests, maintained by the annual removal of above-ground biomass, and hay meadows were considered more appropriate controls (similar to the method used by Edwards & Crawley 1999) than exclosures where no vegetation was removed. The latter approach, although common (Milchunas *et al.* 1992; Kerley *et al.* 1993; Damhoureyeh & Hartnett 1997), would have led to forest formation inside exclosures in our habitat.

We used the random amplified polymorphic DNA (RAPD) technique to estimate the number and spatial distribution of genotypes within four populations of *V. album* that had been grazed for many human generations by domestic cattle and four that had been annually mown during the same general time span. We expect differences between pastures and meadows to be primarily caused by selective avoidance and therefore promotion of such unpalatable species (O'Connor 1994; Gough & Grace 1998; Augustine & McNaughton 1998), although trampling and dung deposition may also play a role. Mechanisms may be suggested by differences in the spatial distributions of genets.

The genetic structure of plant populations is usually related to the species' breeding system, but such information is not available for any species of *Veratrum* (Mulligan & Munro 1987). To gain insight into the long-term effects of grazing we (i) determined the predominant breeding system of *V. album* and asked (ii) whether the spatial and genetic population structure differs between long-term grazed and mown populations and (iii) what mechanism is most likely to be responsible for any differences observed.

Methods

SPECIES

Veratrum album L. is typically found in natural grasslands above the tree line, in manmade grasslands below the tree line, and in open woodlands, and is frequently restricted to moister areas. Other, very similar looking species of this genus occupy the same niche in various parts of the northern hemisphere. Alkaloid poisoning following intake of large quantities of *V. album* may result in death of cattle or deformations in fetuses of a

Table 1 Characteristics of eight *V. album* populations of which the spatial and genetic population structure was determined

Name	Code	Type	Altitude (m a.s.l.)	Location	Shoot density (50 m ⁻²)	No. shoots sampled
Col des Mosses	C	Mown	1360	46°25' N, 7°07' E	77	51
Monts Chevreuils	M	Grazed	1580	46°26' N, 7°05' E	64	48
Les Granges	G	Mown	1320	46°27' N, 7°09' E	102	51
La Braye	O	Grazed	1480	46°27' N, 7°08' E	88	50
Lac des Jones	L	Mown	1200	46°31' N, 6°57' E	133	49
Dent de Lys	D	Grazed	1460	46°31' N, 7°00' E	119	48
Les Rosalys	R	Mown	1180	46°31' N, 6°58' E	56	52
Teyssachaux	T	Grazed	1360	46°32' N, 6°59' E	106	52

range of domestic animals (Binns *et al.* 1972; Mulligan & Munro 1987). Plants are normally avoided by large herbivores, except late in the season, when alkaloid concentrations in the above-ground plant parts decrease (Schaffner 1994) and grazing damage may be observed. Growth commences immediately after snowmelt (mid-April, beginning of May) and most shoots die completely off in the first 2 weeks of August, well before the end of the brief growing season. Although exact data are not available, it probably takes about 40 years to reach flowering size (Hess *et al.* 1967). Each year the vertical rhizome produces one pre-formed apical shoot, which extends to *c.* 60 cm tall and consists of up to 15 leaves on a 'stem' made of the leaf sheaths. Adult plants flower every 4–8 years, when a true stem of up to 2 m tall bears an inflorescence with up to 1000 flowers, each of which may produce *c.* 100 seeds. Flowering is synchronized within and between populations from the Swiss Alps to the French Pyrenees and years when 5–15% of the shoots in a population flower are usually followed by 4 or more years when hardly any inflorescences can be observed (Doree 1988; D. Kleijn, personal observations). After flowering, the apical meristem dies and one to three lateral buds grow out to produce next years' shoots. Thus, unlike many other clonal plants, neither sexual nor vegetative reproduction can occur until a shoot has flowered. As the vertical rhizome progressively decomposes from its base, branching will eventually produce a number of separate clone fragments.

BREEDING SYSTEM EXPERIMENT

The breeding system is not known for any species of *Veratrum*, and a pollination experiment was therefore carried out from July to August 1999 in a *V. album* (hereafter *Veratrum*) population near Les Verrieres in the South Jura Mountains, on an experimental farm of the Federal Station for Agronomic Studies, Changins. On each of five plants, separate side-axes of the inflorescence were assigned to one of four treatments: (i) 'no pollination', flowers were emasculated and bagged with fine-meshed cloth bags to prevent pollination; (ii) 'experimental selfing', flowers were emasculated, self-pollinated by hand, then bagged; (iii) 'experimental outbreeding', flowers were emasculated, pollinated by

rubbing dehiscing anthers of plants growing several metres away onto stigmas, and bagged; and (iv) 'open pollination', flowers were neither emasculated nor bagged. On 20 July 1999, five large, almost open, flower buds on each side axis were selected and the remaining flowers and buds were removed. Flower buds were emasculated, or manipulated in a similar fashion (control) without removing any flower parts (usually resulting in some damage to the petals). Where appropriate, flowers were pollinated on 27 July. On 26 August, when fruits were fully developed, plants were taken to the laboratory for determination of the number of fruits and seeds.

POPULATION STRUCTURE STUDY: SITES AND SAMPLING PROCEDURE

Four hay meadow populations of *Veratrum* were located in the Swiss Pre-Alps. Potential confounding effects of local climatic conditions were minimized by selecting a pasture population as close as possible to each of the rarer meadow populations, but they were too far apart to be considered true pairs (Table 1). The species compositions of these pastures and hay meadows are nevertheless very similar; 90% of species found in one also occur in the other (Schläpfer *et al.* 1998), suggesting that the abiotic conditions are not dissimilar. Furthermore, preliminary results of a parallel study show that plant size and abundance of *Veratrum* are not correlated with nutrient status of the soil in these unfertilized grasslands (D. Kleijn & H. Müller-Schärer, unpublished data). Meadows tended to be moister than the pastures, which occurred on steeper slopes at slightly higher altitudes. Exceptional high winter snowfall meant that pastures were only grazed from the beginning of June until the beginning of October 1999. The vegetation, with the exception of *Veratrum* and a few other unpalatable species, was kept short throughout the season, and even *Veratrum* suffered light to moderate damage late in the season in some pastures. Hay meadows were mown once a year, all after 15 July. Meadow populations were usually somewhat smaller than those in pastures (with the exception of population C) but contained at least 3000 shoots; the size of the largest populations was impossible to estimate as they spread over many hectares of mountain slopes.

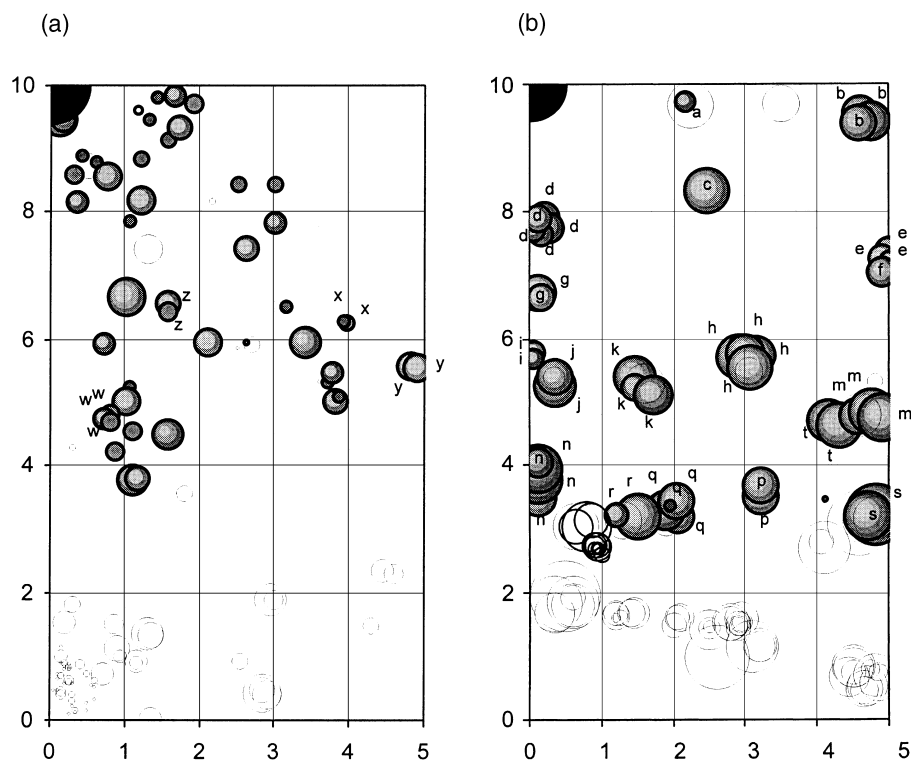


Fig. 1 The distribution and size of *Veratrum album* shoots in 5×10 m plots in (a) a hay meadow (population L) and (b) a pasture (population D). The size of each circle represents dry weight of each shoot (solid quadrant in the upper left corner = 30 g dry weight). Shading indicates shoots for which genotype (RAPD-phenotype) was determined and those with the same letter have the same genotype, all others being unique.

From 13 to 25 July 2000 (before any of the meadows were cut), a 5×10 m plot was laid out within each population in patches that had, at first sight, similar shoot densities (Table 1). In each plot, all *Veratrum* shoots were mapped and cut at ground level. In the laboratory a small leaf sample was taken for DNA extraction (see below) and all shoots then were dried for 48 h at 80 °C and weighed.

RAPD: PROCEDURE

Samples of leaf tissue were cut from a single green, uninfected leaf on each shoot, rinsed with distilled water, snap frozen in liquid nitrogen and freeze-dried for at least 24 h. To determine the genotypic identity of a contiguously located subset of shoots we selected the 50 shoots in each plot which had the highest y-coordinates. A few of these samples did not produce reliable RAPD fingerprints and their omission from the analyses led to some unfingerprinted shoots being present in the fingerprinted area (Fig. 1). Freeze-dried samples were placed in 2.0-mL Eppendorf tubes containing a glass pearl and ground to a fine powder using a shaking mill. DNA was subsequently extracted from the powder following a modified version of the method of Rogers & Bendich (1988) as described by Steinger *et al.* (1996). In short, the ground samples were mixed with CTAB buffer containing 5% mercaptoethanol and incubated at 60 °C. The mixture was extracted twice with chloroform, after which the DNA was precipitated with

isopropanol. The precipitate was washed twice with ethanol, dried and re-suspended in distilled, sterilized water.

Amplification reaction solutions (25 µL) contained DyNAzyme reaction buffer, 100 µM each of dATP, dCTP, dGTP, dTTP, 0.2 µM primer (Operon Technologies Inc., Alameda, California, USA), 0.25 units DyNAzyme polymerase (Finnzymes Inc., Espoo, Finland), and approximately 20 ng DNA (as determined by fluorimeter readings). The reactions took place in polycarbonate microtiter plates with the reaction solution covered by a drop of mineral oil. The samples were incubated in a DNA thermocycler (MJ Research Inc., Watertown, Massachusetts, USA; model PTC100) programmed for 1 min at 93 °C, followed by 45 cycles of 30 s at 92 °C, 30 s at 36 °C and 90 s at 72 °C, and, finally, 5 min at 72 °C. Amplified DNA fragments were separated by electrophoresis on 1.5% agarose gels and visualized by ethidium bromide staining. Gels were digitized on a UV table with a video camera-based imaging system (Biorad Laboratories, Segrate (Milan), Italy; model Gel Doc 2000).

In a first series of tests 54 10-base primers (Operon, kits A, AB and E) were screened for reproducible amplification patterns using replicate DNA extracts from eight leaf samples. Sixty primer-sample combinations were amplified in duplicate as a further check on the reproducibility of the observed DNA-bands. Nine primers that produced 19 reliable polymorphic bands (primers A6, A7, A8, A16, A17, AB11, AB16, AB20,

E17) were selected for RAPD analysis of the field samples.

RAPD: DATA ANALYSIS

Polymorphic bands were scored as present or absent. The presence/absence data were used to identify clones and examine patterns of genetic similarity among shoots. We assume that RAPD band patterns were scored without error and that shoots with different patterns belong to different genotypes (and shoots with the same RAPD-phenotype belong to the same genotype) and therefore use the term genotype rather than the more strictly correct RAPD-phenotype. In terms of clonal plant ecology, a single shoot refers to a ramet (as it is capable of independent growth) and all shoots with the same genotype belong to a single genet. Genotypic diversity for each population was first determined as the proportion of shoots that were distinguishable on the basis of their RAPD-phenotype, i.e. the number of clones detected (G) divided by the sample size (N). A second diversity index was also derived as D , the complement of the Simpson index corrected for finite sample size, i.e. $D_G = 1 - \sum n_i(n_i - 1) / (N(N - 1))$, with n_i being the number of shoots of genotype i and N the total number of shoots in the sample (Pielou 1969). D_G ranges from 0 in populations composed of a single genotype to 1 in populations where each sampled shoot represents a different genotype.

The genetic structure of the eight populations was also examined using the Tanimoto distance (Deichsel & Trampisch 1985). The genetic distance between two shoots was calculated by SGS software (Degen 2000) as $0 \leq D_{ij} = 1 - w_{ij} / (w_{ij} + y_i + y_j) \leq 1$, where w_{ij} represents the number of polymorphic bands that are shared by shoots i and j , and y_i and y_j are the number of bands present uniquely in shoots i and j , respectively. The spatial genetic structure of the population was calculated as the mean genetic distances between all pairs of shoots belonging to a given spatial distance class (eight classes increasing in size from 0.5 to 4.0 m). If the spatial genetic structure is random, then the genetic distance between pairs of shoots is independent of their geographical distance. To test for differences in genetic distance between grazed and mown populations, we performed a one-way ANOVA on the mean D_{ij} -values (ln-transformed) of each distance class, with grazing as the main factor.

ANALYSIS OF THE SPATIAL DISTRIBUTION OF SHOOTS

The distribution of the shoots was examined for spatial autocorrelation by means of Spatial Analysis by Distance IndicEs (SADIE, Perry 1995). The SADIE software for maps uses an algorithm that moves individual shoots in such a way that they fill the allowable space (in our case the 5×10 m plots) in a regular fashion. This process is iterated until the arrangement converges

and the distance to regularity ($Dist_{obs}$) is then computed from a comparison of the initial and final arrangements. Random permutations of the spatial distribution of the n points are subsequently used to calculate the distance to regularity based on a random distribution of the shoots ($Dist_{rand}$). An index of aggregation, I_p , is then obtained from the ratio of observed to permuted values of $Dist$. P_p , calculated as the number of simulated distributions with a $Dist$ greater than $Dist_{obs}$, is used to test whether the observed distribution deviates significantly from a random distribution of the shoots. For a more extensive explanation and discussion of the method, see Perry (1995). These calculations were made for each population and in all tests we used 200 permutations. To examine the effect of scale on the spatial distribution of the shoots, we calculated I_p and P_p for increasingly smaller plots, nested within the main plot. For this purpose we divided the plots in half each time we examined a lower scale-level, the range of scales being 5×10 m to 0.625×1.25 m. To avoid selecting subplots that contained hardly any shoots, and because averaging of all subplots proved to be too computational-intensive (the lowest scale had 256 subplots) we chose, at each lower level, the subplot that contained most shoots. A single exception was population L, which contained a patch of 53 one- and two-leaved plants (< 0.5 g). We excluded this atypical patch in the analysis by taking the opposite half of the 5×10 m plot and then proceeding as previously explained. Analysis with and without the patch showed that patterns revealed by the two analyses were qualitatively the same, but less pronounced in the analysis that included the 'seedling' patch. To examine differences in aggregation between grazed and mown populations, we ln-transformed the I_p -values and, as with the genetic distance index, performed a one-way ANOVA with grazing as the main factor.

Results

BREEDING SYSTEM

Veratrum is effectively a cross-pollinating species. Unpollinated flowers produced no seeds or fruits and self-pollinated flowers very few (Table 2). The latter may represent the presence of some pollen from other plants, rather than true selfing. Cross-pollination by hand resulted in levels of seed and fruit set comparable with open pollination (Table 2). Field observations had already suggested that *Veratrum* flowers are adapted to enhance cross-pollination. Nectar is excreted at the base of the petals of the protandrous flowers and this attracts flies (Diptera), which brush against the shedding anthers or, later, the receptive stigmas.

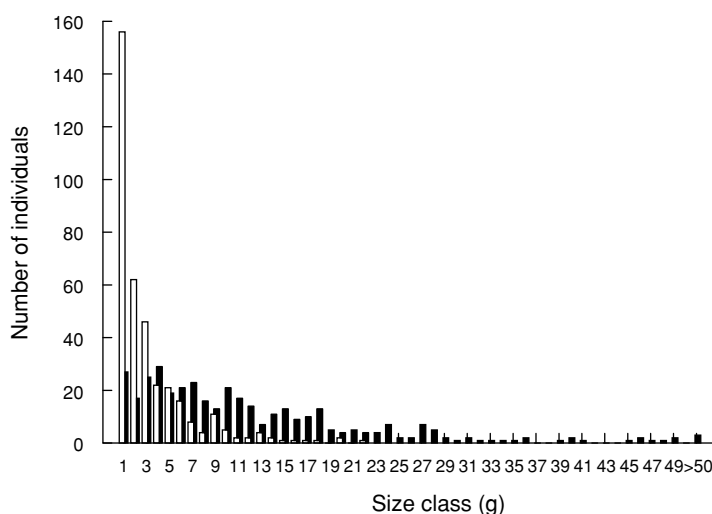
As most seeds are the result of cross-pollination, genetic variation between clones will be large, and the same RAPD-phenotype is unlikely to be found in two different clones.

Table 2 Effect of pollination treatment on fruit and seed production. Mean per plant (five flowers per treatment on each of five plants). Different subscripts indicate significant differences in mean numbers between treatments (ANOVA followed by LSD-test, $P < 0.05$)

Pollination treatment	No. fruits	No. seeds	Seeds/fruit
Emasculated, bagged, not pollinated	0.0 ^b	0.0 ^c	*
Emasculated, bagged and self-pollinated	1.0 ^b	3.4 ^b	3.0
Emasculated, bagged and cross-pollinated	4.0 ^a	87.4 ^a	23.1
Control	4.4 ^a	65.0 ^a	15.2

Table 3 Effect of grazing on *Veratrum* shoots in 5×10 m plots (four populations per grassland type). Data were analysed by one-way ANOVA, biomass data were ln-transformed, and percentage data angular transformed prior to analysis. Plants were sampled in 1999, a year with significant flowering

	Mean shoot mass (g)	Mean number of shoots (50 m^{-2})	Percentage of flowering shoots	Total above-ground biomass (g.m^{-2})
Mown	3.1	92	0.33	4.9
Grazed	11.4	94	4.30	21.82
<i>F</i>	10.91	0.01	8.80	15.49
<i>P</i>	0.016	0.925	0.025	0.008

**Fig. 2** Pooled size distribution of *Veratrum album* shoots in four mown (open bars, $n = 368$) and four grazed populations (solid bars, $n = 376$).

CHARACTERIZATION OF HAY MEADOW AND PASTURE POPULATIONS OF *VERATRUM*

Although we chose plots with similar mean shoot densities, other characteristics contrasted sharply between meadows and pastures (Table 3). Mean shoot dry weight, total biomass production and proportion flowering were significantly higher in grazed than in mown populations. Meadow populations had high numbers of small plants and few large plants (Fig. 2), whereas the size distribution of shoots was much more even in pasture populations, although still skewed to the right. *Veratrum* seedlings look like grass shoots and remain extremely small for at least the first growing season. Seedlings grown for a month under favourable laboratory conditions reached a size of 0.0038 g dry weight including roots ($n = 10$, SE = 0.0012; U. Treier, unpublished results). As no shoots collected in the field were

less than 0.01 g dry weight it is likely that most seedlings were missed. Clonally propagated shoots, however, can rapidly attain sizes well over 1 g, and can therefore easily be distinguished from seed-derived juveniles. Figure 2 clearly shows that seed-derived juvenile plants were much more common in mown than in grazed populations.

SPATIAL STRUCTURE OF *VERATRUM* POPULATIONS

At the 50 m^2 plot level shoots were highly aggregated in both the mown and the grazed populations (Table 4, Fig. 1). The index of aggregation decreased with size of the subplots in mown populations with a random distribution of shoots at the smallest scale ($I_p < 1$), but shoots remained highly clumped in grazed populations ($I_p < 2$ at all scales). Despite considerable variation

Table 4 Index of aggregation (I_p) of *Veratrum album* populations in pastures and hay meadows at a range of scales. $I_p = 1$ indicates a random distribution, $I_p > 1$ indicates spatial aggregation, $I_p < 1$ indicates regular distribution. * $P_p < 0.05$, ** $P_p < 0.01$; both based on permutation tests. To examine differences between grazed and mown populations, ln-transformed indices were analysed by one-way ANOVA

	Plot size (m ²)						
	50	25	12.5	6.25	3.125	1.56	0.78
I_p : mown populations							
C	1.08	1.07	1.19	1.59**	1.43	0.95	0.81
G	2.06**	1.91**	0.96	1.04	1.03	1.07	1.43
L	4.83**	1.66**	1.48*	1.03	0.90	0.95	0.84
R	1.80**	2.09**	1.80**	1.49**	1.60*	1.38*	1.18
Mean	2.44	1.68	1.36	1.29	1.24	1.09	1.07
I_p : grazed populations							
M	1.65*	1.46**	1.66**	1.49**	1.64*	2.20**	2.14**
O	2.99**	1.91**	1.99**	1.64**	1.53*	1.38*	1.27
D	2.73**	1.81**	1.85**	2.32**	2.71**	2.22**	2.13**
T	2.61**	2.25**	1.96**	1.58**	1.94**	2.53**	2.01**
Mean	2.50	1.86	1.87	1.76	1.96	2.08	1.89
$F_{1,6}$ (grazed vs. mown)	0.20	0.46	5.78	4.22	6.05	16.28	9.76
P	0.671	0.524	0.053	0.086	0.049	0.007	0.020

Table 5 Genotypic diversity of *Veratrum album* populations in pastures and hay meadows (both $n = 4$). G/N is the proportion of distinguishable genets, D_G is Simpson's index of diversity. Mean clone size (number of shoots) was ln-transformed, indices were angular-transformed followed by one-way ANOVA

	Clone size	G/N	D_G
<i>V. album</i> populations			
Mown	1.1	0.907	0.996
Grazed	2.1	0.539	0.962
$F_{1,6}$	10.58	12.10	11.53
P	0.017	0.013	0.015

between populations of the same type (for example, at 0.78 m², the grazed population O was atypically random), grazed populations were significantly more aggregated than mown populations at c. 3 m² or below (Table 4).

GENETIC STRUCTURE OF *VERATRUM* POPULATIONS

When represented by G/N , genotypic diversity was substantially lower in grazed than in mown populations where the proportion of distinguishable genets was only slightly over 0.5, indicating that almost 50% of the shoots were of clonal origin (Table 5). Less than 10% of shoots in mown populations originated from vegetative propagation and genotypes here consisted of one to three shoots, compared with up to 11 in pastures (Fig. 3). Figure 1 shows that clones form compact clumps of shoots. As this species does not form elongated rhizomes, but propagates by fragmentation of a vertically branched rhizome, different ramets of the same clone are expected to grow close to each other. Maximum distance between two shoots of the same

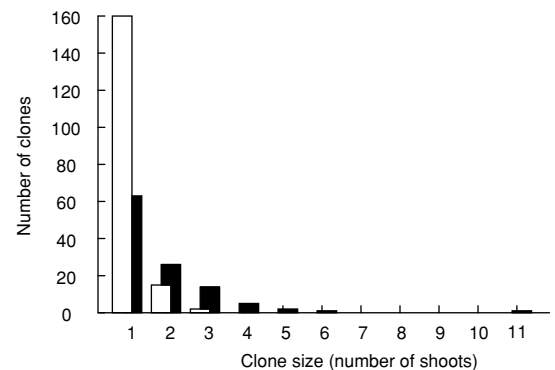


Fig. 3 The number of *Veratrum album* genotypes with the same number of shoots pooled over the four mown populations (open bars) and four grazed populations (closed bars).

clone was 13 cm and 75 cm in mown and grazed populations, respectively (data not shown). Almost all clumps consisted of a single genotype, suggesting that small-scale aggregation of shoots is not due to adult *Veratrum* shoots (which are avoided by cattle) acting as a nursery or safe site for its seedlings (which may be grazed as they are too small to be selectively avoided by cattle).

Although D_G was very high in both population types, this measure of genotypic diversity was, at least statistically, significantly lower in grazed populations (Table 5). The high genotypic diversity in pastures, despite the large proportion of clonal offspring, is probably caused by the relatively small mean size of clones (Table 5). Genetic distance did not vary with spatial separation except for pairs of shoots in pasture populations below 0.5 m, which were more similar than in meadows (Fig. 4). This is most probably caused by the more prolific clonal propagation in grazed populations, which is only apparent within the small scale of the clumps.

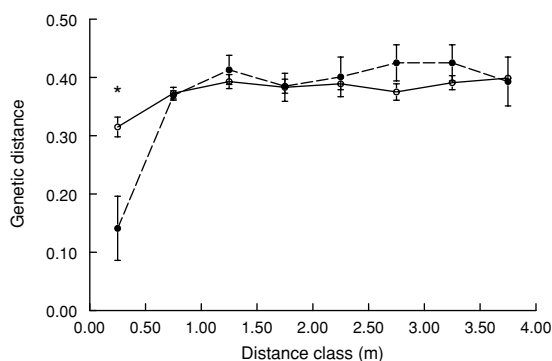


Fig. 4 The mean genetic distance (Tanimoto index) between pairs of shoots growing at increasing distances from each other in mown (open circles) and grazed (closed circles) populations of *Veratrum album*. Asterisks indicate significant differences ($P < 0.05$) between mown and grazed populations within a distance class (analysed by one-way ANOVA).

Discussion

The spatial and genetic population structure of *Veratrum* in grazed pastures was strikingly different from that in hay meadows. Pasture populations, which had relatively few juvenile plants, consisted mainly of large shoots, which often grew in tight genotypically uniform clumps. Although 50% of the shoots appeared to be of clonal origin, a modest, but substantial proportion of the shoots produced inflorescences. In contrast, hay meadow populations had many juvenile plants. Most shoots were medium sized and widely spaced: only *c.* 10% had clonal origins, but these again grew close to each other. Hardly any shoots produced inflorescences.

PLANT SIZE IN RELATION TO HABITAT CONDITIONS

In these grasslands, succession is arrested by cutting or grazing and plants in these communities must therefore have specific adaptations to tolerate the regular loss of most above-ground biomass. Only in the pastures are plants selectively removed, promoting some species at the expense of others (Allen *et al.* 1984; Anderson & Briske 1995; Augustine & Frelich 1998). In pastures, therefore, *Veratrum* shoots (i) grow with reduced competition for light (surrounding vegetation removed by livestock), (ii) have a longer growing season (hay is typically cut 2–3 weeks prior to their natural senescence) and (iii) are able to re-translocate resources from the shoot to the rhizome before senescence. Plants in pastures, therefore, probably have larger below-ground reserves as well as larger shoots. Although dung and urine contribute to increased soil fertility in pastures, plant size and abundance of *Veratrum* is generally not correlated with nutrient status (D. Kleijn & H. Müller-Schärer, unpublished data) and are thus unlikely to contribute to the observed larger shoot size.

SEXUAL REPRODUCTION IN RELATION TO HABITAT CONDITIONS

The higher proportion of flowering shoots in pasture populations may be a direct result of their larger shoots. Flowering frequency in many long-lived plant species is positively correlated with plant size (Cipolinni & Stiles 1991; Cunningham 1997), and as neither seedling recruitment nor clonal propagation can occur until *Veratrum* plants have flowered, may be a major determinant of their population structure. However, the number of juvenile plants (i.e. those below 1 g, Fig. 2) is much lower in pastures than meadows despite the latter being harvested before seed set. Seeds from plants in adjacent forest edges and around isolated trees within meadows, which usually escape being cut, are probably responsible for these seedlings. Large herbivores often have positive effects on seedling recruitment by opening up the vegetation through grazing and creating gaps through trampling (Bakker 1987; Silvertown & Smith 1988; Bullock *et al.* 1994; Edwards & Crawley 1999; but see Milchunas *et al.* 1992), although the resulting seedlings are more likely than adult plants to be killed by grazing (Crawley 1983). Additionally, the abiotic conditions experienced by seedlings when the vegetative cover has been removed (particularly temperature and soil moisture) are often much harsher, resulting in increased seedling mortality rates (Ryser 1993). Our results suggest that seedling establishment and survival was extremely low under grazed conditions so that, despite many seeds being produced, few seedlings persist in the population.

SPATIAL STRUCTURE IN RELATION TO GRASSLAND TYPE

In nature, spatial aggregation of plants is the rule rather than the exception (Hutchings 1997). At the whole plot level (50 m²), both the pasture and the hay meadow populations were significantly aggregated (Table 4), but only in pastures did *Veratrum* demonstrate a typical small-scale clumping of shoots. Within these clumps shoot bases were generally spaced within 20 cm of each other and usually consisted of a single genotype (Fig. 1). Seed dispersal patterns and factors that control seedling establishment (e.g. herbivory, competition, environmental stress) and contribute to large scale aggregation do not therefore appear to be significantly different between grazed and cut populations but clonal expansion of established plants that leads to small-scale aggregation was predominantly restricted to pasture populations. Vegetative reproduction in many clonal plant species is positively correlated with plant size (e.g. Mendez & Obeso 1993; Verburg *et al.* 1996; Mulder & Ruess 1998; Lenssen *et al.* 2000) and the larger mean size of *Veratrum* in pastures may lead to higher clonal growth rates (through the development of more lateral buds per rhizome after flowering).

as well as to a higher frequency of clonal propagation (due to more frequent flowering).

Such clonal growth may reduce the risk of trampling: solitary shoots, even when they are large, run a high risk of being trampled, but cows generally walk around dense patches of mature *Veratrum* shoots (D. Kleijn, personal observations). Even for wind-dispersed herbaceous species, most seeds fall at the foot of the mother plant (Portnoy & Willson 1993; Jongejans & Schippers 1999) and one might therefore expect *Veratrum* to act as a nurse plant (see Callaway 1995) for seedlings developing within the shoot clump. However, most clumps consist of a single genotype, suggesting that seedlings rarely establish here and this may be due to negative feedback on seedlings in close proximity to parents, mediated by soil pathogens (Packer & Clay 2000).

GENETIC STRUCTURE IN RELATION TO GRASSLAND TYPE

The genotypic diversity of pasture populations, as expressed by Simpson's index D_G , was marginally, albeit significantly, lower than meadows. The observed values (0.962 vs. 0.996) are within the range (0.10–1.00) found by Ellstrand & Roose (1987) across studies of clonal diversity in species that rarely reproduced by seeds. Species, such as *Veratrum*, that regularly re-establish from seeds as well as by means of ramets usually have a D_G close to 1 (e.g. Chung & Epperson 1999). The relatively small difference in D_G between grazed and mown populations, despite the former having 40% more shoots of clonal origin, may be caused by the limited distance between sister ramets and the modest size of individual clones. No single clone was able to dominate and spread throughout the population. Genetic structure was only observed in pasture populations and then only in that the mean genetic similarity of shoots was higher for those growing within 0.5 m of each other (Fig. 4), reflecting the presence of clone patches. These occur only in pastures, and at other distances the populations were similar. The lack of genetic structure, other than that due to clonal growth, may be the result of the breeding system: *Veratrum* is an obligate cross-pollinator (Table 2) and the pollinating flies may cover large distances (Sheppard 1994), resulting in an even distribution of genes within the population. At the population level (rather than at the 5×10 m plot level) it is therefore unlikely that significant differences existed in the genotypic diversity between grazed and mown populations.

MECHANISMS INFERRED FROM THE OBSERVED PATTERNS AND ECOLOGICAL IMPLICATIONS

The divergence in spatial and genetic population structure can only be explained by a shift in the mode of reproduction in the two population types. This occurs

predominantly through seeds in meadows, while clonal reproduction accounts for half of the population growth in pasture, which, in the long term, would result in the observed divergence, given the life history of *Veratrum*. Our field study corroborates predictions based on computer simulation models by Barkham & Hance (1982) and Watkinson & Powell (1993), who found that the population structure of clonal plant species is predominantly determined by the ratio of seedling to ramet recruits.

The most plausible cause for the shift in mode of reproduction is that shoots in pastures are selectively avoided by cattle. This results in shoots having a longer growing season with less competition from the surrounding vegetation than would have been the case were they palatable or were biomass removed irrespective of palatability (as in hay meadows or when burnt). Some variability could, however, be due to the different environmental conditions experienced by pasture and meadow populations. Kerley *et al.* (1993) observed a similar shift towards clonal growth in *Yucca elata* in the presence of grazers, which was attributed to the high herbivore-induced mortality of small plants and this process probably also plays an important role in the population dynamics of *Veratrum*. In an early isoenzyme-study, Gray *et al.* (1979) compared two populations of the coastal grass *Puccinellia maritima* and found that mean clone size was larger under grazed than undisturbed conditions. Selection against genotypes that flower abundantly (flower heads are generally grazed) and in favour of those that exhibit prostrate vegetative spread was given as the most plausible mechanism for the effect of grazers. A recurring theme seems to be that clonal propagation is generally less affected than seedling recruitment by large herbivores so that increased grazing intensities generally result in a larger mean clone size. Further studies addressing this point directly are required before any robust hypothesis can be formulated, however.

The results of the present study cannot distinguish between possible causes of the reduced seedling recruitment and a demographic experiment is therefore being undertaken to separate the direct (removal of biomass) and indirect (e.g. drought stress) effects of grazing. A combination of descriptive studies on population structure, such as the present one, and experimental/demographic studies on key processes in the population dynamics are clearly needed to unravel fully the effects of herbivores on long-lived plant species, as well as the mechanisms responsible.

In central Europe, *Veratrum* occurs primarily in mountain grasslands that, in this densely populated region, are one of the few remaining extremely species-rich habitats. Were grazing to lead to an increased dominance of this tall species it might have negative consequences for the conservation of these ecosystems, but *Veratrum* obtains only local dominance even in pastures. This may be because grazing promotes vegetative growth and clonal expansion of established

plants, but probably also reduces the chance of long-range seedling establishment. This could explain why *Veratrum* is usually found as dense populations but is not found at all in other habitats with apparently suitable environmental conditions. In the central European Alps a number of other unpalatable species occupy the same general niche and have similar life histories to *Veratrum* (e.g. *Cirsium* spp., *Rumex alpinus*, *Senecio alpinus*). Processes similar to those that correlate with the success of *Veratrum* in pastures may very well explain the dominance of these species in grazed communities. More generally, numerous studies have documented the increase in abundance of unpalatable species in response to (increased levels of) grazing (Grant *et al.* 1985; Bauer 1990; Brown & Stuth 1993; O'Connor 1994; Augustine & McNaughton 1998; Gough & Grace 1998; Edwards & Crawley 1999). Studies on population structure and population level responses to herbivory have mainly concentrated on palatable species (e.g. Kerley *et al.* 1993; Bastrenta *et al.* 1995; Augustine & Frelich 1998). Considering the importance of grazing in steering species change of entire ecosystems, detailed information on population level responses of unpalatable (dominant) plant species would clearly help us understand and predict these changes.

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