

Effect of grazing and mowing on the clonal structure of *Elytrigia atherica*: a long-term study of abandoned and managed sites

Vliv pastvy a kosení na klonální strukturu porostů druhu *Elytrigia atherica*: dlouhodobá studie různých typů obhospodařování

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Dedicated to the memory of Leoš Klimeš

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Physical disturbance by large herbivores can affect species diversity at the community level and concurrently genetic diversity at the species level. As seedling establishment is rarely observed in clonal plants, short-term experiments and demographic studies are unlikely to reveal the response of clonal plants to disturbances. A long-term (30-year) field experiment and the availability of molecular markers allowed us to investigate the clonal structure of populations of *Elytrigia atherica* subjected to different management regimes. The long-term field study provided us with five replicated blocks that had been subjected to three different management regimes, grazing by cattle, mowing and abandonment. In this study we examined the effects of herbivore grazing and mowing on clonal richness and genetic diversity of populations in salt marshes using multilocus microsatellite genotypes. In addition, phenotypic traits and spatial positions of *E. atherica* ramets were determined for 20 samples in a 5 × 10 m plot in each of the blocks. Abundance and phenotypic traits were affected by the management regimes, resulting in a higher abundance in abandoned fields and plants having shorter and narrower leaves in managed fields. Biomass removal did affect the clonal structure of populations and increased the genetic diversity compared to that in abandoned fields. However, no distinct difference was found between the two management regimes, mowing and grazing. Although seedling recruitment has rarely been observed, the present study shows that such rare events have occurred within the populations studied. Thus, molecular tools can greatly increase our understanding of vegetation dynamics and processes within populations growing under different conditions.

Key words: clonal diversity, genetic diversity, herbivores, management regime, disturbance, salt marsh, species diversity, Wadden Sea

Introduction

Plant community structure, composition and dynamics can be greatly affected by land-use practices such as mowing or grazing by large herbivores (Kleijn & Steinger 2002, Ehrlén et al. 2005). Studies have shown that grazing, trampling and/or mowing can alter succession, plant species diversity, structural heterogeneity and productivity (Ritchie & Olf 1999).

Large herbivore-exclusion experiments have shown that herbivores can promote or reduce species diversity (Bakker 1989, Crawley 1997, Olf & Ritchie 1998). Through biomass removal and trampling gaps are created in the existing vegetation. This mechanism reduces competition, enhances seedling recruitment and colonization and hence local plant species diversity (Collins & Barber 1986). Other studies have shown that herbivores can have a devastating effect on seedling survival (Bakker & De Vries 1992, Milchunas et al. 1992, Crawley 1997). There are a few detailed studies of the effect of grazing and mowing on population structure (Kölliker et al. 1998, Kotanen & Bergelson 2000, Bühler & Schmid 2001, Bockelmann 2002). These studies focus on monitoring the fate of individual plants and describing differences in phenology and morphology (Watkinson & Powell 1993). However, much more can be learned about the effect of disturbance on population structure.

Most clonal plant species combine vegetative and sexual reproduction and as a consequence have complex population structures. Secondly, grazing or mowing may have different effects on the reproductive strategies of plant species (Fernandez et al. 1992, Kerley et al. 1993). Some plant species respond to grazing by compensatory growth and producing more side shoots (ramets) (Mcnaughton 1983, Wallace et al. 1985), whereas other species may decrease or increase their seed production. As mentioned above, seedling recruitment may either be increased or limited by grazing. For many clonal species disturbances, such as grazing or fires, are needed for seedling establishment (Eriksson 1989) as these disturbances reduce the competition from the established vegetation (Eriksson 1997). When seedling establishment is rarely observed in clonal plant populations, the general assumption is that the population is dominated by a few large clones. However, genetic studies on clonal diversity have shown the contrary (Richards et al. 2004, Scheepens et al. 2007). Thus, analysis of spatial and genetic population structure is an important tool for inferring the most likely processes involved in any observed effect of disturbance (Kleijn & Steinger 2002).

Only a few studies have dealt with the effect of disturbance by management on the clonal diversity of plant populations (Kleijn & Steinger 2002, Reisch & Scheitler 2009). Both studied the effect of disturbance by comparing sites with different land use. Due to large distances between and the different environments at the sites, the treatments could not be treated as a pair. Experimental studies on the processes determining clonal structure and diversity are lacking, in particular at small spatial scales of several m², and at a large temporal scale of decades.

Salt marshes along the European Wadden Sea have been grazed since their establishment, around 1000 years A.C. Over the past few decades the management regimes of the salt marshes of the Wadden Sea have changed. The cessation of grazing of the salt marsh has led to the dominance of a few species, and hence a decrease in the number of plant communities and plant species (Van Wijnen & Bakker 1997). The native wheat grass *Elytrigia atherica* is one of the species that has been increasing in abundance and dominating as a result of this change in the management regime. The palatable, tall grass *E. atherica* is capable of both vegetative and sexual propagation. In 1972 a long-term field experiment was initiated to study the effects of management on species composition and diversity (Bakker 1989). Three different management regimes, i.e. grazing by cattle, mowing and abandonment were established and monitored for nearly 30 years in replicated blocks. While in the mown fields only biomass was removed, grazing by cattle additionally introduced trampling effects. The study by Bakker (1989) showed that abandon-

ment of the fields resulted in a decrease in species diversity and increase in the abundance of *E. atherica* (Fig. 1).

This long-term field experiment and the availability of molecular markers provide a unique opportunity to study the effects of management regimes on the genetic structure of a clonal plant species. We used the polymorphism displayed by microsatellite markers to identify genets and determine the clonal structure and genetic diversity. The aim of the study is to address whether the clonal structure of *E. atherica* populations is affected by the different management regimes. As biomass removal may reduce competition and enhance seedling recruitment, a higher clonal diversity is expected in the grazing and mowing treatments. Mowing was carried out only once a year, whereas cattle remove biomass throughout the season by repeated grazing and trampling, hence leading to small-scale heterogeneity and more diversity in the grazed plots. This brings about the following questions: (i) How do the different management regimes (abandoned, grazing and mowing) affect the clonal diversity in a clonal palatable grass species? (ii) Do different management regimes affect species diversity in a way similar to their affect on the clonal structure and genetic diversity of *E. atherica*?

Methods

Study species

Elytrigia atherica (Link) Kerguélen ex Carreras Mart (*Triticeae: Poaceae*) is a tall-growing grass that reproduces clonally via rhizomes and sexually (Bockelmann et al. 2003). The species is native to salt marshes and occurs along the North Atlantic Coast from Northern Portugal to Southern Denmark. It is wind-pollinated, as are most *Poaceae*. The main dispersal units are spikelets, which bear up to five seeds (A. C. Bockelmann, personal observation.). Spikelets have no obvious dispersal mechanism. The species is hexaploid ($6x = 2n = 42$; Stace 1995, Hess et al. 1998). *Elytrigia atherica* is usually out-crossing (Dewey 1983) but is also self-compatible (Bockelmann 2002).

Study site

The present study was conducted on a salt marsh on the Dutch Frisian island of Schiermonnikoog (53°30'N, 6°10'E). The study site is situated in the western part of the salt marsh and has an area of approximately 32 ha. This part of the salt marsh was grazed by young cattle until 1958, after which the salt marsh was abandoned. In 1972, the grazing regime (from beginning of May until end of October) was resumed in a fenced area with a stocking rate of 1.3 to 1.7 head of cattle/ha. Five distinctive plant communities, initially dominated by *Juncus maritimus*, *Festuca rubra/Armeria maritima*, *Elytrigia atherica*, *Festuca rubra/Limonium vulgare* and *Artemisia maritima*, respectively, were selected to study the changes in vegetation (Bakker & De Vries 1992). Within each plant community, concurrently with the introduction of the cattle, a mowing experiment was initiated in the un-grazed salt marsh adjacent to the fence (Bakker 1978, Bakker 1989). In each plant community there were three plots (5 × 10 m), adjacent to each other, assigned to cattle grazing, mowing in June and abandonment. The distance between the five blocks, each with three treatment plots, varied between 100 m and 400 m. Changes in the vegetation were recorded annually

by scoring the cover of all species occurring in permanent quadrats of 2×2 m in each plot from 1972 onwards. In total 15 permanent quadrats, three treatments per plant community, and five distinct plant community vegetation relevés, based on the decimal scale (Londo 1976) were annually recorded. Plant species nomenclature follows Van der Meijden (2005).

The sampling in the present study was conducted in November 2000. In each plot the tiller density was sampled three times by randomly placing a 10×10 cm frame within the plot and counting the tillers within this frame. Within each plot phenotypic traits, such as shoot length, width and length of upper leaf were measured for ten randomly selected tillers. Secondly, in each plot 20 tillers of *E. atherica* were randomly selected for genetic analysis. The location (xy-coordinates) of these tillers was measured and drawn on a map. Young leaf tissue of these mapped tillers was collected and preserved for DNA extraction by drying with silica-gel. In total 300 tillers were selected for genetic analysis.

Genetic analysis

The distinction between genets and ramets within the sampled tillers of *Elytrigia atherica* was based on the microsatellite polymorphism displayed at four marker loci. For this study, four microsatellite markers originally designed for two other species of *Poaceae*, *Elymus caninus* (Sun et al. 1998) and *Triticum aestivum* (Röder et al. 1998), were used (Table 1). Detailed information on cross-species modification of these microsatellite primers is described by Bockelmann (2002) and Bockelmann et al. (2003). DNA was extracted following the CTAB method (Doyle & Doyle 1987). The extracts were purified and subjected to PCR with fluorescence-labelled primers following (Bockelmann et al. 2003). DNA samples were stored at -20°C until further analysis. The PCR products of primer pairs WMS6 and WMS44, and primer pairs of WMS2 and ECGA89, respectively, were pooled. The products were visualized on an ABI-377 automated sequencer (Applied Biosystems), together with an internal size standard (Gene ScanTM-350 ROXTM, Applied Biosystem). To check the repeatability of the results positive and negative controls were always used. When errors were detected or weak DNA signal and/or banding pattern observed, amplifications were repeated. Samples with weak banding patterns for one of the four primers were not included in subsequent analyses.

Data analysis

As *E. atherica* is a hexaploid species a codominant analysis for microsatellite markers could not be applied (Bockelmann et al. 2003, Scheepens et al. 2007). Therefore, the banding patterns of the microsatellite markers for each individual sample were interpreted as multilocus fingerprints. To ensure repeatable and reliable genotype assignment, the scoring of polymorphic fragments was automated (Bonin et al. 2004). Polymorphic fragments were scored as present or absent, resulting in a multilocus genotype per ramet, using the software packages GENESCAN and GENOTYPER (Applied Biosystems). Secondly, the scoring data of identical multilocus genotypes, multilocus genotype pairs with a genetic distance of only two bands and a random subset of genotypes were manually checked. In the manual check not only the presence of fragments was scored, but also the amplification intensity of each fragment. As *E. atherica* is a polyploid species, the amplified fragments show different intensities. The chance of overestimating the number of clones was reduced by incorporating amplification when assigning identical multilocus genotypes.

Table 1. – Microsatellites primer pairs of *Triticum aestivum* (WMS) and *Elymus caninus* (ECGA) used to assess multilocus genotypes of *Elytrigia atherica*.

Primer name	Repeats in original species	Repeats in <i>E. athericus</i>	Fragment size range (bp)	No. of fragments
WMS 2	(CA) ₁₈	(CA) ₁₀	185–295	5
WMS 6	(GA) ₄₀	(GA) ₁₅	138–164	12
WMS 44	(GA) ₂₈	no data	115–147	9
ECGA 89	(GA) ₁₇	(GA) ₁₂ G(GA) ₄	190–208	7

The probability of a particular multilocus genotype is given by the product of the single locus genotype probabilities in random mating conditions $P_{\text{gen}} = \prod p_i$, in which p_i is the frequency of fragment presence at each locus in the observed multilocus genotype (Sydes & Peakall 1998). The probability of obtaining $n-1$ more copies of that genotype by chance is given by $(P_{\text{gen}})^{n-1}$, where n is the number of times the genotype was observed (Mandel 2010).

Clonal richness for each plot was determined as the number of genets detected (G) divided by the sample size (N) (Kleijn & Steinger 2002). Simpson's index for diversity corrected for finite samples was used as a measure of genotypic diversity per plot, $D_G = 1 / \sum n_i(n_i - 1) / N(N - 1)$, where n_i is the number of individuals in the i -th genotype, N is sample size (Ellstrand & Roose 1987, Kleijn & Steinger 2002, Arnaud-Haond et al. 2007). Distance between identical genet pairs was measured using geographical information software (ArcGis 9.3).

For each phenotypic trait, the mean and standard error of the randomly selected tillers per plot were calculated. We applied analysis of variance to test for effects of treatment (abandoned, grazed and mown) on phenotypic traits and clonal structure using the software program SPSS 16 (2007) for statistical analyses. Transformations were used in the analysis in order to adhere to assumptions of normality and homogeneity of variance, but the measured values were used to produce the figures. The response data was first tested in a general linear model, in which the different plots were fixed and the blocks (initial plant community) random factors. When the block effect was not significant, this random factor was emitted from the analysis.

Results

Effect of management on plant community

Although the experiment was initially (1972) set up in different plant communities, there were no significant differences between the communities in species diversity (one-way ANOVA, $F = 0.41$, $df = 25$, $P = 0.8$) or standing biomass in 2000 (one-way ANOVA, $F = 1.901$, $df = 25$, $P = 0.148$). However, the different management regimes resulted in changes in the initial communities. In 1972 *E. atherica* occurred only in one block, the *E. atherica* plant community. After 15 years *E. atherica* rapidly started to dominate the abandoned plots. This expansion resulted in the establishment of an *E. atherica* plant community, independent of the initial plant community, in all the abandoned plots (one-way ANOVA, $F = 15.151$, $df = 13$, $P = 0.001$, post hoc Tukey) (Fig. 1A). Over the years the species diversity (number of species in 4 m²) diverted between the treatments. In 2000 the abandoned plots

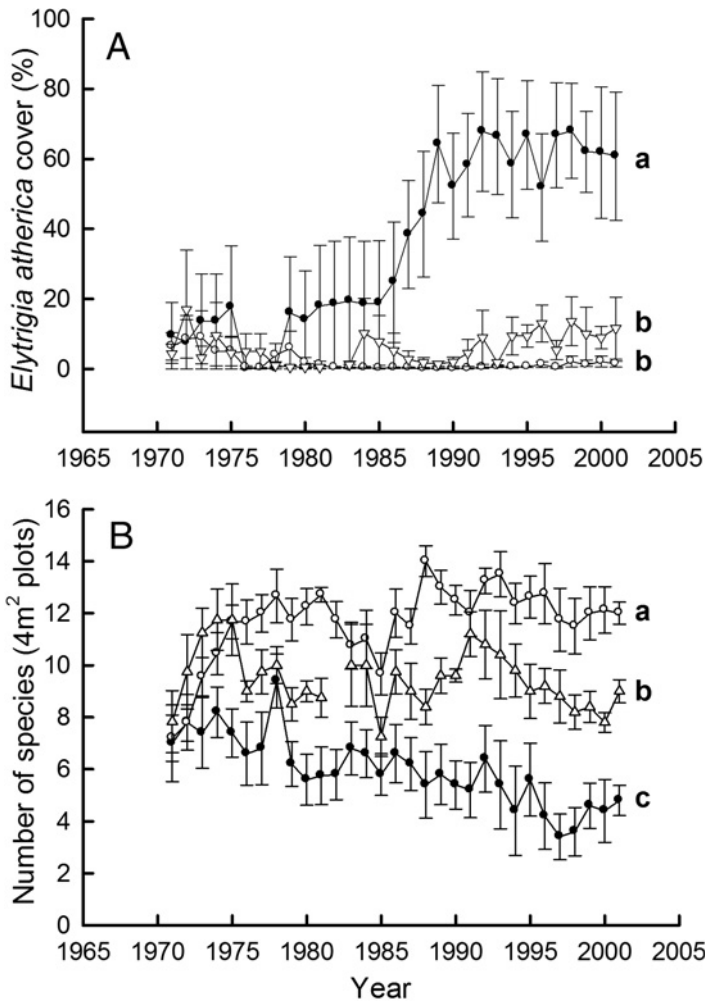


Fig. 1. – Effect of management on (A) the cover of *Elytrigia atherica* and (B) number of species in plots that were abandoned in 1972 (closed circles) and permanent quadrats (2 × 2 m) that were grazed by cattle (open circles) or mown (triangles). Different letters indicate significant ($P < 0.05$) differences in 2000.

had the lowest species number and the grazed plots the highest number of species (one-way ANOVA, $F = 14.258$, $df = 13$, $P = 0.001$, post-hoc Tukey) (Fig. 1B). The initial plant community, as a random factor, had no significant effect on species diversity

Effect of management on plant morphology

In all management regimes *E. atherica* was present at widely varying densities. In the abandoned plots *E. atherica* formed monocultures, whereas in the grazed and mown plots its abundance (as percentage cover) was often $< 1\%$ (Fig. 1A). The ramet density of *E. atherica* was at least twice as high in the abandoned plots than the managed plots, how-

ever not significantly so (Table 2). There were significant differences in the phenotypes recorded in the abandoned versus grazed and mown treatments. The mown and grazed plants had significantly shorter shoots and narrower leaves than the ramets on unmanaged plots (Table 2). Except for the length of the primary leaf, the initial plant community, as a random factor, had no significant effect on plant morphology.

Table 2. – Phenotypic traits of *Elytrigia atherica* (n = 10) and tiller density (n = 3) in each management regime (N = 5). Data were analyzed using one-way ANOVA, density was log-transformed and leaf width square-root transformed. Different letters indicate significant (P < 0.01) differences between management regimes based on post hoc Tukey HSD test.

Management regime	Abandoned	Grazed	Mown	F-value	df
Density (no. ramets/100 cm ²)	52.1±9.6 ^c	17.7±8.2 ^a	13.1±2.7 ^b	3.534	14
Leaf number per tiller	5.12±0.25 ^a	4.28±0.24 ^a	4.64±0.19 ^a	3.372	14
Shoot length (cm)	53.4±2.4 ^a	23.6±3.1 ^b	23.8±1.9 ^b	50.767	14
Leaf width (cm)	4.45±0.14 ^a	3.33±0.15 ^b	3.13±0.13 ^b	25.330	14

Effect of management on clonal structure

A total of 38 different sized fragments were recorded among the four primer pairs that could be reliably scored when comparing duplicates and all were polymorphic. The highest number of polymorphic fragments was found at locus WMS 6, including 15 fragments, locus WMS44 showed nine fragments. The primer pairs WMS2 and ECGA89 amplified seven fragments each. The number of fragments scored per sampled ranged between 6 and 18. On average 11 amplified fragments were scored per sample. Among 296 samples a total of 236 different multilocus genotypes were found, resulting in a grand clonal diversity of 0.77. A histogram of genetic distances among multilocus genotypes shows that

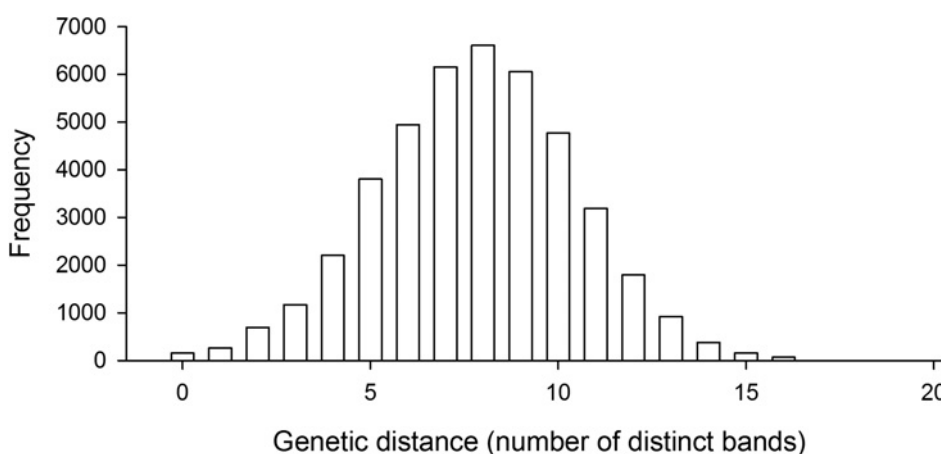


Fig. 2. – Frequency distribution of band differences among 296 different *Elytrigia atherica* multilocus genotypes based on four microsatellite loci.

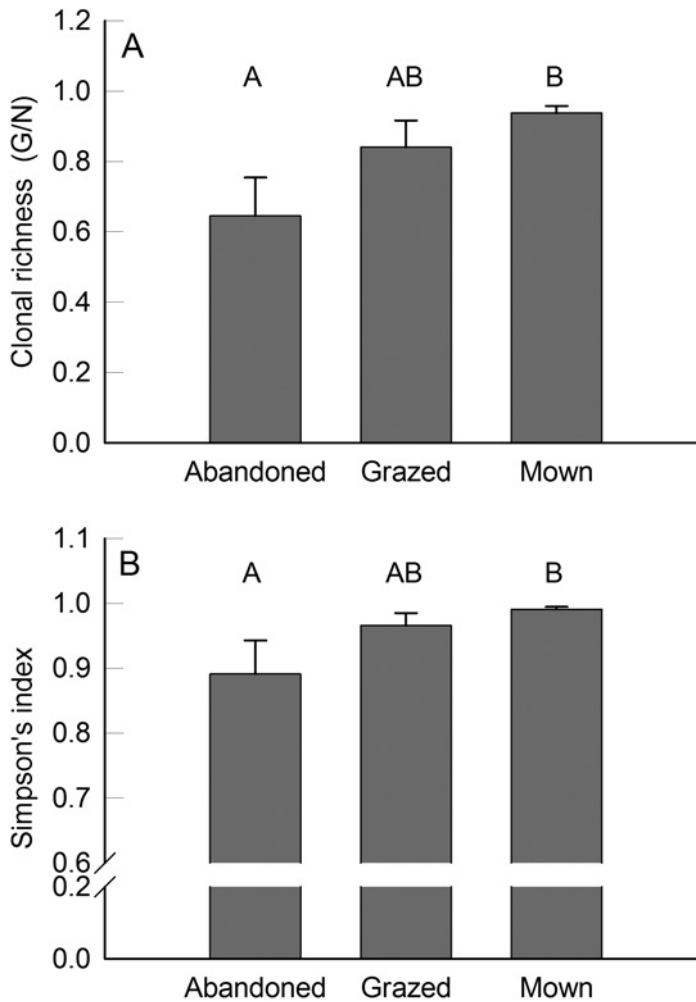


Fig. 3. – Effect of management regime, i.e. abandoned, grazed or mown, on the clonal diversity of *Elytrigia atherica*, measured in terms of (A) clonal richness and (B) the Simpson diversity index. The bars represent the means for 20 tillers and standard errors for the results for the different management regimes, and those with different letters differ significantly ($P < 0.05$), based on Mann-Whitney U tests.

band differences between multilocus genotypes are large (Fig. 2). At least 95% of the multilocus genotype pair combinations differed in more than two fragments. This 95% interval was also used as the threshold genetic distance. Multilocus genotype pairs that differed in only two fragments were checked and assigned manually.

The probability P_{gen} values ranged from 0.03 to 1.3×10^{-6} . The values of P_{dgen}^{n-1} , the probability of drawing more than one copy of a certain multilocus genotype from a randomly mating population, were always lower than 0.05 (range from 0.03 to 2.3×10^{-20}). This implies that multiple occurring multilocus genotypes were always considered to originate from clonal reproduction. The clonal richness, represented by G/N, was significantly

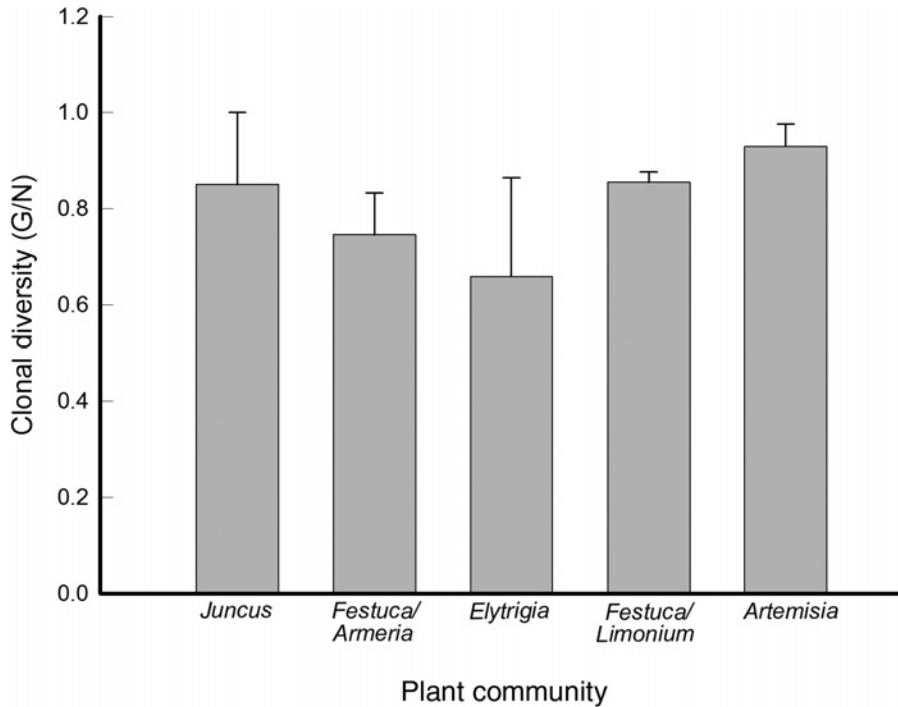


Fig. 4. – Comparison of the clonal richness of *Elytrigia atherica* in different plant communities. The bars represent mean clonal richness and standard error recorded in the three management regimes within each plant community. There are no significant differences in clonal richness in the different plant communities.

higher in the managed than the abandoned plots (Kruskal-Wallis, $\chi^2 = 6.54$, $df = 2$, $P = 0.04$, Fig. 3A). In the abandoned plots more than 40% of the shoots were of clonal origin, whereas in the managed plots less than 20% originated from vegetative propagation. In comparison with the other plant communities, the G/N was relatively low in the *E. atherica* plant community, which had a G/N ranging from 0.75 to 0.93. However, the difference between the plant communities was not significant. In the abandoned *E. atherica* plot there were only five genets in the 19 ramets (G/N = 0.66, Fig. 4).

The Simpson's diversity index was very high in all management regimes. It was significantly higher in the mown than the abandoned plots (Kruskal-Wallis, $\chi^2 = 6.06$, $df = 2$, $P = 0.05$). Grazing resulted in an intermediate Simpson's diversity (Fig. 3B). For both clonal richness and Simpson's diversity, the grazed plots were intermediate to the abandoned and mown plots (Fig. 3A, B). The initial plant community, as a random factor, had no significant effect on genetic diversity or richness parameters.

Sometimes clones grew intermingled within plots and between plots (Fig. 5). Twice, a pair of identical genets was found to occur in two fields with different management regimes. The distance between one of these pairs was 41m, which occurred in the abandoned and grazed *E. atherica* community plot. This pair had a genotype with low likelihood of occurring by chance ($P_{\text{gen}} = 0.0028$). Most pairs of identical genets (n) were found in the abandoned plots, $\approx 11\%$ of all possible sample-pair combinations (N), with an

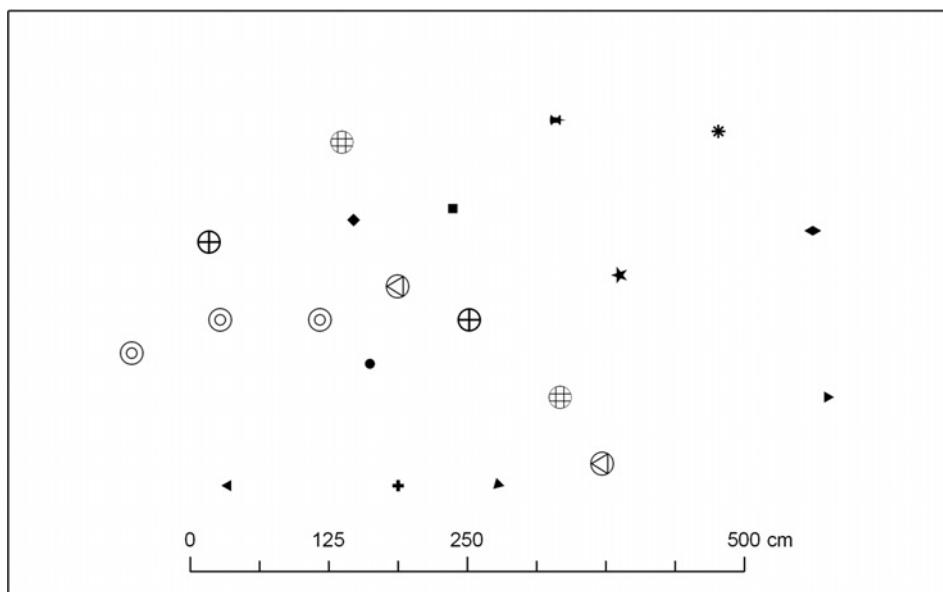


Fig. 5. – Distribution of clones in the abandoned plot in the *Festuca/Armeria* community, an example of intermingled plots. Open circles with identical inner symbols represent ramets with identical genets. Closed symbols represent unique ramets.

average clone size of 1.87 ramets per genet (Table 2, Fig. 6). In the grazed and mown, 4% and 1%, respectively, of the sample-pairs had an identical genotype (Fig. 6). The lowest average distance between identical genet pairs was found in the mown plots (Table 3, Fig. 6). The observed minimum distance between identical genet pairs was 36 cm.

Table 3. – Effect of management regime on the clonal structure of *E. atherica* based on 20 tillers per management regime. Clone size is the mean number of ramets per genotype (\pm S. E.). The mean distance between identical genotypes is expressed in terms of clone distance (m) and the maximum mean distance between two identical genotypes in each treatment. Data were analyzed using a Kruskal-Wallis test, for which the χ^2 and df are given; different letters row-wise indicate significant ($P < 0.05$) differences.

Management regime	Abandoned	Grazed	Mown	χ^2	df	P
Clone size	1.87 \pm 0.47 ^a	1.23 \pm 0.12 ^{ab}	1.07 \pm 0.02 ^b	6.54	2	0.04
Mean clone distance (m)	2.19 \pm 0.21	3.90 \pm 0.88	1.25 \pm 0.41	5.23	2	0.07
Maximum mean distance between identical genets (m)	3.50	8.80	3.15	–	–	–

Discussion

Consistent with the initial hypothesis, we found higher clonal richness (as G/N) in mown plots compared to abandoned plots. Although the expectation was that the abandoned and managed plots would differ, the grazed plots were intermediate between abandoned and

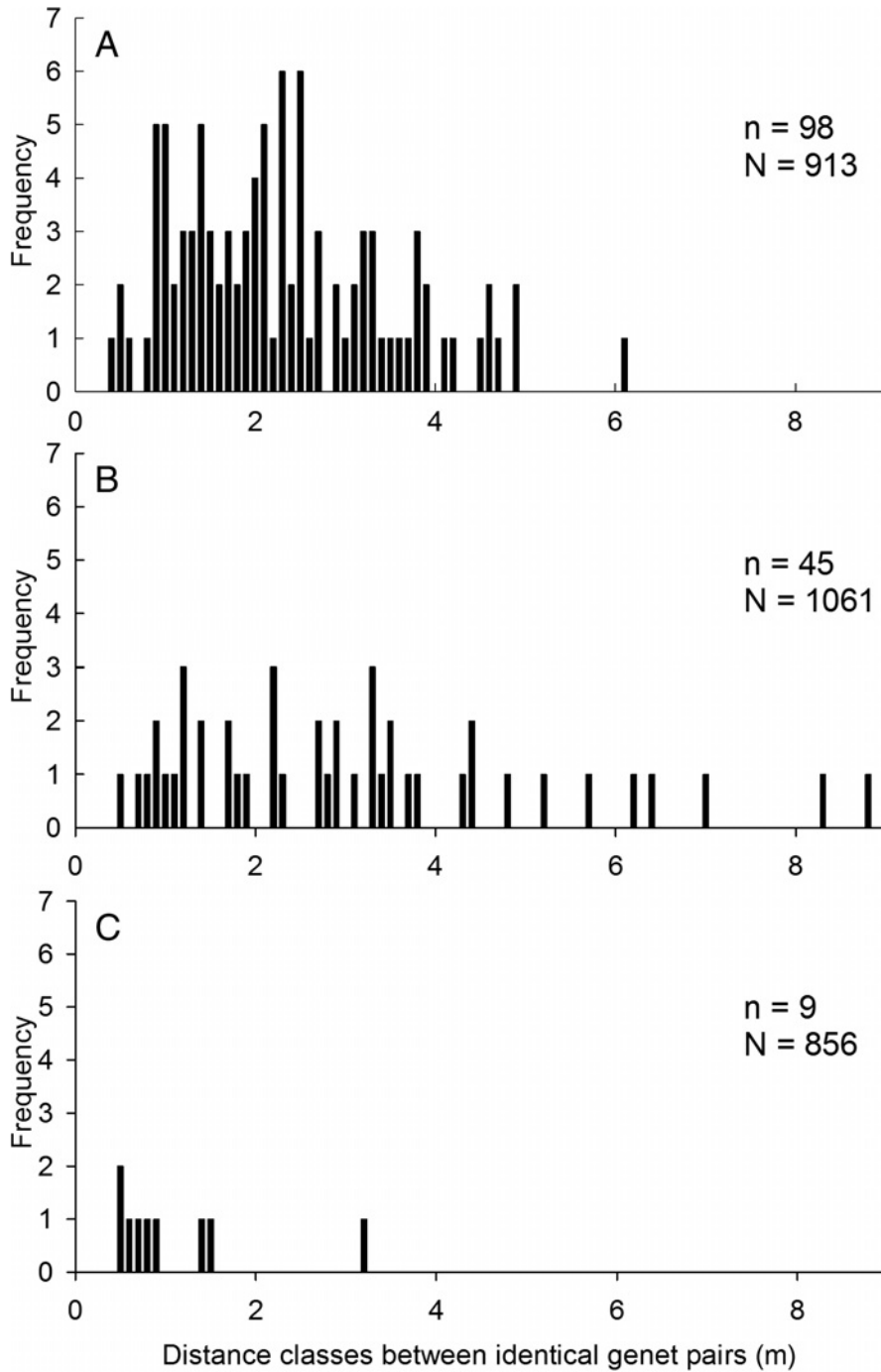


Fig. 6. – Frequency distribution of distances between ramets of the same genet in (A) abandoned, (B) grazed and (C) mown plots, with the number of identical genet-pairs indicated by n and total sample-pair combinations possible by N.

mown plots for both clonal richness and in terms of Simpson's diversity index. Apparently, the clonal structure is not only affected by biomass removal by herbivores, but possibly also by other mechanisms.

Clonal diversity

Elytrigia atherica dominated the abandoned plots, while this species was rare in grazed and mown plots. As *E. atherica* is a tall grass, its high abundance corresponds to a high standing biomass throughout the year with concomitant competitive suppression of seedling recruitment. The apparent differences in canopy height between the managed and abandoned plots could have resulted in the difference in clonal structure between the mown and abandoned plots. Two possible processes could explain the low clonal diversity in the tall and dense canopy of the *E. atherica* community in the unmanaged plots. First, the high standing biomass could limit seedling recruitment, as competition for light will be high (Wilson & Tilman 1991, Bakker & De Vries 1992). In many clonal plant species seedling recruitment is highly dependent on disturbance, e.g. removal of above-ground biomass (Watkinson & Powell 1993, Eriksson & Jakobsson 1998). Secondly, disturbance, like mowing and grazing, may enhance the transport of seeds and bulbils (Mouissie et al. 2005, Reisch & Scheitler 2009). Thirdly, with the increase in the number of individuals there is an increase in intraspecific competition among adult plants (Bengtson 2003), which may result in a loss of genets and decrease in clonal diversity (Gray et al. 1979, Watkinson & Powell 1993). Low genetic diversities resulting from intraspecific competition are recorded for various species at different timescales: *Lolium perenne* (Mcneilly & Roose 1984), *Calamagrostis epigejos* (Lehmann 1997), *Spartina alterniflora* (Travis & Hester 2005) and *Elytrigia atherica* (Scheepens et al. 2007).

Contrasting effects of grazing and mowing on clonal diversity

A higher clonal diversity was expected in the grazed than in the mown areas. Mowing took place once a year, whereas cattle may remove biomass throughout the season by repeated grazing and trampling leading to small scale heterogeneity and hence a greater species diversity in the grazed plots. However, in the present study there was no difference in species diversity in the different management regimes. Kleijn & Steinger (2002) found different effects of mowing and grazing on the clonal structure of *Veratum album*. The low clonal diversity in grazed populations of *V. album* is most likely due to large herbivores avoiding this toxic species and as a consequence enhancing its vegetative reproduction. *Elytrigia atherica* is a palatable species for cattle therefore selective removal did not play a role in this study.

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). The percentage of bare soil was highest in the grazed parts of the salt marsh site and seed germination of lower marsh species is higher in the grazed plots (Bakker & De Vries 1992). Gap size can affect seedling establishment (Silvertown & Smith 1988, Watt & Gibson 1988). The amount of bare soil ranged between 20% and 40% in both grazed and mown treatments at this salt-marsh study site (Bakker & De Vries 1992). Therefore, it is unlikely that gap size affected the clonal structure in the present study.

Despite the higher germination recorded in the grazed plots, Bakker & De Vries (1992) found a higher seedling survival in mown than in grazed plots at the present study site. In grasslands, small-scale disturbances may enhance germination but not necessarily seedling survival (Eriksson 1997). In *Trifolium repens*, disturbance enhances germination but the survival of seedlings was higher in undisturbed vegetation (Barrett & Silander 1992). Kuijper *et al.* (2004) show that when neighbouring plants are removed the survival of seedlings of *E. atherica* was lower than when subject to competition from neighbouring plants. The mown regimes are low disturbance areas, as the vegetation was cut and removed only once annually, whereas cattle grazed the site from early May till end October. The destruction of seedlings by trampling might explain the higher survival in the mown than the grazed plots in several instances, especially in the heavily grazed *Festuca-Limonium* community (Bakker & De Vries 1992).

Effect of management on clonal structure

Watkinson & Powell (1993) predict using a simulation model, that genet density of *Ranunculus repens* is higher in areas of high seedling recruitment whereas inequality of clone size tends to be higher in areas of low seedling recruitment. Kerley *et al.* (1993), Kleijn & Steinger (2002) and Liston *et al.* (2003) record a larger clone size in the presence of grazers. A greater clone size in undisturbed populations is also recorded for *Calamagrostis epigejos* (Lehmann 1997) and *Lolium perenne* (Mcneilly & Roose 1984). Reusch (2006) records enhanced seedling recruitment in response to physical disturbance in *Zostera marina*. Conversely, in populations of *Puccinellia maritima* the mean clone size is larger in grazed than undisturbed habitats and seedling establishment more frequent in un-grazed marsh, unlike that recorded in our study (Gray *et al.* 1979). In the present study we observed an inequality of clone size when there was little disturbance and intraspecific competition as on the abandoned marsh. The single mowing event reduces competition and creates a window of opportunity for seedling recruitment. The presence of large numbers of small sized genets and the highest total number of genets in the mown plots support this. Although disturbance by cattle create windows of opportunity for seedling recruitment the continuous nature of the disturbance reduces the chance of successful recruitment. This is supported by the few but large clones in the grazed areas, where the greatest distance between identical clones was observed (Fig. 4). In conclusion, this study indicates that management does have an effect on clonal structure, but there is a threshold. Disturbance can enhance seedling recruitment but too much disturbance will enhance clonal dispersal.

Conclusions

Few experimental studies have focused on the effect of disturbance on genet dynamics, recruitment or genotypic diversity, and none that we are aware of deal with medium temporal (i.e. 30 years) and spatial (100 m²) scales as in the present study. The studies discussed above mention an effect of disturbance on genet dynamics and recruitment. Only in some of these studies was a significant effect of management on the genetic diversity and differentiation between populations recorded. Lehman (1997), Liston *et al.* (2003) and Reusch (2006) did find any differences in clonal structure of populations subject to disturbance. However, this effect was also weak, which will be reflected in clonal diversity.

Despite, the large demographic differences recorded in populations of *Brachypodium pinnatum* (Schlapfer & Fischer 1998), *Piptochaetium napostaense* (Tomas et al. 2000) and *Festuca idahoensis* (Matlaga & Karoly 2004) caused by different grassland managements, the clonal diversity or genetic variation was unaffected by the treatments. The time-scale of these manipulative studies, ranging from three years (Reusch 2006) to 64 years (Matlaga & Karoly 2004), could be for some populations or species too short to observe such changes in clonal diversity or genetic differentiation. Other studies suggest that the morphological differentiation between plants with different grazing histories seems to be the outcome of a phenotypically plastic response of adapted genotypes.

In the *E. atherica* populations studied there was a clear difference in clonal structure and clonal diversity in response to different management over a period of nearly 30 years. Using molecular tools to study the clonal structure of populations can help us understand and unravel the dynamics and processes in populations subject to different conditions. As hypothesized biomass removal does affect the clonal structure of populations and increases the genetic diversity. However, no distinct differences were recorded for the two management regimes, mowing and grazing.

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Souhrn

Fyzické narušení porostu způsobené velkými herbivory může ovlivnit druhovou bohatost na úrovni společenstva a genetickou variabilitu na úrovni populace. Protože semenné obnovování bývá u klonálních rostlin pozorováno vzácně, krátkodobé experimenty a demografické studie nemusí být dostatečné pro zjištění vlivu narušení na populace klonálních druhů rostlin. Třicetiletý terénní experiment a znalost molekulárních markerů nám umožnily zkoumat klonální strukturu porostů druhu *Elytrigia atherica*, vystavených různým typům obhospodařování. V experimentu byly použity tři varianty (pastva dobytka, kosení a ponechání ladem) v pěti opakováních. Pro vyhodnocení vlivu různého obhospodařování na klonální strukturu a genetickou bohatost populací na slanisku jsme využili microsatelity. Navíc jsme zjišťovali fenotypické vlastnosti a prostorové uspořádání prýtlů zkoumaného druhu z 20 vzorků, odebraných na plochách 5 × 10 m v každém pokusném bloku. Abundance a fenotypické znaky byly ovlivněny typem obhospodařování. Na opuštěných plochách rostl druh s vyšší abundancí, na obhospodařovaných plochách měly rostliny užší a kratší listy. Odstraňování biomasy neovlivnilo klonální strukturu populací a zvýšilo genetickou diverzitu. Oproti očekávání nebyly nalezeny rozdíly ve zkoumaných parametrech mezi kosením a pastvou. I když uchycení semenáčků bylo pozorováno jen zřídka, v průběhu experimentu k němu docházelo. Molekulární metody představují vhodný nástroj k hlubšímu porozumění dynamice vegetace v různých podmínkách.

References

- Arnaud-Haond S., Duarte C. M., Alberto F. & Serrao E. A. (2007): Standardizing methods to address clonality in population studies. – *Mol. Ecol.* 16: 5115–5139.
- Bakker J. P. (1978): Changes in a salt marsh vegetation as a result of grazing and mowing: a five year study of permanent plots. – *Vegetatio* 38: 77–87.
- Bakker J. P. (1987): Grazing as a management tool in the restoration of species-rich grasslands. – *Proc. Koninklijke Nederlandse Akademie Van Wetenschappen Series C* 90: 403–430.
- Bakker J. P. (1989): Nature management by grazing and cutting. – *Kluwer Acad. Publ.*, Dordrecht.

- Bakker J. P. & De Vries Y. (1992): Germination and early establishment of lower salt-marsh species in grazed and mown salt-marsh. – *J. Veg. Sci.* 3: 247–252.
- Barrett J. P. & Silander A. J. (1992): Seedling recruitment limitation in white clover (*Trifolium repens*: *Leguminosae*). – *Am. J. Bot.* 79: 643–649.
- Bengtson B. O. (2003): Genetic variation in organisms with sexual and asexual reproduction. – *J. Evol. Biol.* 16: 189–199.
- Bockelmann A. C. (2002): Ordinary and successful: the invasion of *Elymus athericus* in European salt marshes. – PhD thesis, Univ. Groningen, Groningen.
- Bockelmann A. C., Reusch T. B. H., Bijlsma R. & Bakker J. P. (2003): Habitat differentiation vs. isolation-by-distance: the genetic population structure of *Elymus athericus* in European salt marshes. – *Mol. Ecol.* 12: 505–515.
- Bonin A., Bellemain E., Eidesen P. B., Pompanon F., Brochmann C. & Taberlet P. (2004): How to track and assess genotyping errors in population genetics studies. – *Mol. Ecol.* 13: 3261–3273.
- Bühler C. & Schmid B. (2001): The influence of management regime and altitude on the population structure of *Succisa pratensis*: implications for vegetation monitoring. – *J. Appl. Ecol.* 38: 689–698.
- Bullock J. M., Hill B. C., Dale M. P. & Silvertown J. (1994): An experimental study of the effects of sheep grazing on vegetation change in a species-poor grassland and the role of seedlings recruitment into gaps. – *J. Appl. Ecol.* 31: 493–507.
- Collins S. L. & Barber S. C. (1986): Effects of disturbance on diversity in mixed-grass prairie. – *Vegetatio* 64: 87–94.
- Crawley M. J. (1997): Plant-herbivore dynamics. – In: Crawley M. J. (ed.), *Plant ecology*, p. 401–475, Blackwell Sci., Oxford.
- Dewey D. R. (1983): Historical and current taxonomic perspectives of *Agropyron*, *Elymus*, and related genera. – *Crop Sci.* 23: 637–642.
- Doyle J. J. & Doyle J. L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – *Phytochem. Bull.* 74: 11–15.
- Ehrlén J., Syrjänen K., Leimu R., Garcia M. B. & Lehtila K. (2005): Land use and population growth of *Primula veris*: an experimental demographic approach. – *J. Appl. Ecol.* 42: 317–326.
- Ellstrand N. C. & Roose M. L. (1987): Patterns of genotypic diversity in clonal plant-species. – *Am. J. Bot.* 74: 123–131.
- Eriksson O. (1989): Seedling dynamics and life histories in clonal plants. – *Oikos* 55: 231–238.
- Eriksson O. (1997): Clonal life histories and the evolution of seed recruitment. – In: De Kroon H. & Van Groenendael J. M. (eds), *The ecology and evolution of clonal plants*, p. 211–226, Backhuys Publ., Leiden.
- Eriksson O. & Jakobsson A. (1998): Abundance, distribution and life histories of grassland plants: a comparative study of 81 species. – *J. Ecol.* 86: 922–933.
- Fernandez R. J., Nunez A. H. & Soriano A. (1992): Contrasting demography of two Patagonian shrubs under different conditions of sheep grazing and resource supply. – *Oecologia* 91: 39–46.
- Gray A. J., Parsell R. J. & Scott R. (1979): The genetic structure of plant populations in relation to the development of salt marshes. – In: Jefferies R. L. & Davy A. J. (eds), *Ecological processes in coastal environments*, p. 43–64, Blackwell Sci. Publ., Oxford.
- Hess H. E., Landolt E. & Hürzel R. (1998): *Flora der Schweiz und Angrenzender Gebiete, Band 1, Pteridophyta bis Caryophyllaceae*. – Birkhäuser, Basel.
- Kerley G.-I. H., Tiver F. & Whitford W. G. (1993): Herbivory of clonal populations: cattle browsing affects reproduction and population structure of *Yucca elata*. – *Oecologia* 93: 12–17.
- Kleijn D. & Steinger T. (2002): Contrasting effects of grazing and hay cutting on the spatial and genetic population structure of *Veratrum album*, an unpalatable, long-lived, clonal plant species. – *J. Ecol.* 90: 360–370.
- Kölliker R., Stadelmann F. J., Reidy B. & Nosberger J. (1998): Fertilization and defoliation frequency affect genetic diversity of *Festuca pratensis* Huds. in permanent grasslands. – *Mol. Ecol.* 7: 1557–1567.
- Kotaniemi P. M. & Bergelson J. (2000): Effects of simulated grazing on different genotypes of *Bouteloua gracilis*: how important is morphology? – *Oecologia* 123: 66–74.
- Kuijper D. P. J., Nijhoff D. J. & Bakker J. P. (2004): Herbivory and competition slow down invasion of a tall grass along a productivity gradient. – *Oecologia* 141: 452–459.
- Lehmann C. (1997): Clonal diversity of populations of *Calamagrostis epigejos* in relation to environmental stress and habitat heterogeneity. – *Ecography* 20: 483–490.
- Liston A., Wilson B. L., Robinson W. A., Doescher P. S., Harris N. R. & Svejcar T. (2003): The relative importance of sexual reproduction versus clonal spread in an aridland bunchgrass. – *Oecologia* 137: 216–225.
- Londo G. (1976): The decimal scale for relevés of permanent quadrats. – *Vegetatio* 33: 61–64.

- Mandel J. R. (2010): Clonal diversity, spatial dynamics, and small genetic population size in the rare sunflower, *Helianthus verticillatus*. – *Cons. Genet.* 11: 2055–2059.
- Matlaga D. & Karoly K. (2004): Long-term grazing effects on genetic variation in Idaho fescue. – *J. Range Manage.* 57: 275–279.
- Mcnaughton S. J. (1983): Compensatory plant-growth as a response to herbivory. – *Oikos* 40: 329–336.
- Mcneilly T. & Roose M. L. (1984): The distribution of perennial ryegrass genotypes in swards. – *New Phytol.* 98: 503–513.
- Milchunas D. G., Lauenroth W. K. & Chapman P. L. (1992): Plant competition, abiotic, and long-term and short-term effects of large herbivores on demography of opportunistic species in a semiarid grassland. – *Oecologia* 92: 520–531.
- Mouissie A. M., Lengkeek W. & van Diggelen R. (2005): Estimating adhesive seed-dispersal distances: field experiments and correlated random walks. – *Funct. Ecol.* 19: 478–486.
- Olf H. & Ritchie M. E. (1998): Effects of herbivores on grassland plant diversity. – *Trends Ecol. Evol.* 13: 261–265.
- Reisch C. & Scheitler S. (2009): Disturbance by mowing affects clonal diversity: the genetic structure of *Ranunculus ficaria* (*Ranunculaceae*) in meadows and forests. – *Plant Ecol.* 201: 699–707.
- Reusch T. B. H. (2006): Does disturbance enhance genotypic diversity in clonal organisms? A field test in the marine angiosperm *Zostera marina*. – *Mol. Ecol.* 15: 277–286.
- Richards C. L., Hamrick J. L., Donovan L. A. & Mauricio R. (2004): Unexpectedly high clonal diversity of two salt marsh perennials across a severe environmental gradient. – *Ecol. Lett.* 7: 1155–1162.
- Ritchie M. E. & Olf H. (1999): Spatial scaling laws yield a synthetic theory of biodiversity. – *Nature* 400: 557–560.
- Röder M. S., Korzun V., Wendehake K., Plaschke J., Tixier M.-H., Leroy P. & Ganal M. W. (1998): A microsatellite map of wheat. – *Genetics* 149: 2007–2023.
- Scheepens J. F., Veeneklaas R. M., Van de Zande L. & Bakker J. P. (2007): Clonal structure of *Elytrigia atherica* along different successional stages of a salt marsh. – *Mol. Ecol.* 16: 1115–1124.
- Schlapfer F. & Fischer M. (1998): An isozyme study of clone diversity and relative importance of sexual and vegetative recruitment in the grass *Brachypodium pinnatum*. – *Ecography* 21: 351–360.
- Silvertown J. & Smith B. (1988): Gaps in the canopy: the missing dimension in vegetation dynamics. – *Vegetatio* 77: 57–60.
- SPSS (2007): SPSS 16 for Windows. 16.0.1. 2007. – SPSS Inc., Chicago.
- Stace C. A. (1995): Flora of the British Isles. – Cambridge Univ. Press, Cambridge.
- Sun G. L., Salomon B. & von-Bothmer R. (1998): Characterization and analysis of microsatellite loci in *Elymus caninus* (*Triticeae: Poaceae*). – *Theor. Appl. Genet.* 96: 676–682.
- Sydes M. A. & Peakall R. (1998): Extensive clonality in the endangered shrub *Haloragodendron lucasii* (*Haloragaceae*) revealed by allozymes and RAPDs. – *Mol. Ecol.* 7: 87–93.
- Tomas M. A., Carrera A. D. & Poverene M. (2000): Is there any genetic differentiation among populations of *Piptochaetium napostaense* (Speg.) Hack (*Poaceae*) with different grazing histories? – *Plant Ecol.* 147: 227–235.
- Travis S. E. & Hester M. W. (2005): A space-for-time substitution reveals the long-term decline in genotypic diversity of a widespread salt marsh plant, *Spartina alterniflora*, over a span of 1500 years. – *J. Ecol.* 93: 417–430.
- Van der Meijden R. (2005): Heukel's flora van Nederland. Ed. 23 [Heukel's flora of the Netherlands]. – Wolters-Noordhoff, Groningen.
- Van Wijnen H. J. & Bakker J. P. (1997): Nitrogen accumulation and species replacement in three salt marsh systems in the Wadden Sea. – *J. Coastal Cons.* 3: 19–26.
- Wallace L. L., Mcnaughton S. J. & Coughenour M. B. (1985): Effects of clipping and four levels of nitrogen on the gas-exchange, growth, and production of two East African graminoids. – *Am. J. Bot.* 72: 222–230.
- Watkinson A. R. & Powell J. C. (1993): Seedling recruitment and the maintenance of clonal diversity in plant-populations: a computer-simulation of *Ranunculus repens*. – *J. Ecol.* 81: 707–717.
- Watt T. A. & Gibson C. W. D. (1988): The effects of sheep grazing on seedling establishment and survival in grassland. – *Vegetatio* 78: 91–98.
- Wilson S. D. & Tilman D. (1991): Components of plant competition along an experimental gradient of nitrogen availability. – *Ecology* 72: 1050–1065.

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