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Effects of neighbourhood structure and tussock dynamics on genet demography of *Festuca rubra* in a mountain meadow

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Summary

1 We examined whether genet demography in the clonal grass *Festuca rubra* in a mountain grassland in the Krkonoše Mountains (Czech Republic) changes in response to local environment. The data were also used to compare genet recruitment with estimates of genet mortality.

2 We sampled *F. rubra* from four permanent plots in which ramet densities of all species have been recorded at a fine scale for a decade. Identities of 224 ramets were assessed by means of RAPD, yielding 145 different genets, of which most (68%) were found only once. The genet with the highest number of sampled ramets was recorded nine times.

3 By assuming that the probability that two ramets are genetically identical is a function of distance between ramets, we estimated that successful seedling recruitment rates were between 0.15 and 16 genets m^{-2} yr^{-1} depending on the plot. Genet mortality was estimated from ramet mortality using the assumption that ramet mortality was uncorrelated over space. In three of the four plots, genet mortalities fell into the same range as the genet recruitment rates.

4 The total number of genets per unit area must be known to enable determination of per capita recruitment and mortality rates. We developed an estimation technique involving simulation of the sampling process, which yielded values of 231–968 genets m^{-2}. Genet turnover was therefore low (0.1–1% annually) and the high genet diversity is maintained by a very low recruitment.

5 Spatial analysis showed that two ramets were more likely to be genetically identical when they came from a microhabitat where *Festuca* ramet density had recently increased; patches in which *Festuca* ramet density had decreased were more likely to contain ramets from several genets. Expanding tussocks are thus more likely to be composed of ramets of one genet only. Density and biomass of other species in the neighbourhood showed little correlation with the genet structure of *Festuca rubra.*

6 Although ramets of *Festuca rubra* intermingle with other species at a fine scale, the population biology of its genets is driven mainly by the dynamics of its own ramets. Interactions with other species and response to local environments have little effect on genet structure.

Key-words: clonal plants, fine-scale permanent plots, genet demography, RAPD, sampling simulation

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Introduction

For a long time, plant population biology was restricted to the study of populations of non-clonal plants. Until recently, the population biology of clonal plants could only be studied at the ramet level as genets were difficult to identify, and therefore difficult to count or measure [Eriksson 1993; see also Bradshaw (1959), Harberd (1963) and Harberd & Owen (1969) for early attempts to study genet-level population biology of clonal plants]. However, molecular markers now permit identification of individual genets, thus making systematic analysis of genet-level population biology of clonal plants possible (Ellstrand & Roose 1987; Hamrick & Godt 1989; Widén *et al*. 1994).

Study of the demography of genets requires information both on recruitment and mortality of genets, and on total genet population sizes. Molecular data on genet identities (e.g. Steinger *et al*. 1996; Kudoh *et al*. 1999; Pornon *et al*. 2000; Stehlik & Holderegger 2000) can be re-analysed to produce numbers of genets per sample, and, when sampling has been done in a spatially defined way, to gauge distances between ramets belonging to the same genet (an estimate of genet size). Unfortunately, such results only provide very indirect information on demographically relevant parameters.

Genet recruitment frequency is one of the commonest parameters for which molecular marker data can be used. If annual rate of spread, distances between mother and daughter ramets, and ramet recruitment rate are known, then genet recruitment can be calculated from genet numbers and the probability that two ramets belong to the same genet, either by simulations (Watkinson & Powell 1993; Suzuki *et al*. 1999) or by an analytical calculation (Harada *et al*. 1997; Kudoh *et al*. 1999). Although this approach is possible only if it is assumed that genet recruitment and mortality are in equilibrium, it is the first essential step for an understanding of genet demography. Furthermore, if the spatial positions of the sampled ramets are known, genet sizes can be estimated from the greatest distance between ramets with the same molecular identity. This allows the age distribution and mean or maximum age of genets to be determined (Eriksson & Bremer 1993; Steinger *et al*. 1996; Jónsdóttir *et al*. 2000).

Little information has been collected on other key demographic variables, such as total genet numbers and genet mortalities in populations. Information on total number of genets per unit area is rarely available as we generally do not know the relationship between the value obtained from the number of genets found in the sample and the total number of genets per unit area. Most molecular marker studies thus do not yield an estimate of the total number of genets and, as a consequence, they cannot be used to assess the importance of genet recruitment for overall genet dynamics (relative genet turnover or mean genet lifespan). Information on mortality is even more limited. It is therefore still a considerable task for plant population biologists to describe the dynamics of the total genet population of a clonal plant species.

A major research topic in population biology of non-clonal plants has been to examine how the demography of a species varies as a function of the density, history, habitat or microhabitat conditions of its populations (e.g. Gustafsson & Ehrlén 2003; Quintana-Ascencio *et al*. 2003). If the population dynamics of genets is established for a clonal species, a similar research programme should become possible for genet demography. As spatial and/or temporal heterogeneity is ubiquitous in all habitats (Caldwell & Pearcy 1994), there may be fine-scale variation in genet-level demography within the community, or fine-scale differentiation of genotypes (Prentice *et al*. 1995, 2000). However, there is very little information on whether genet-level population biology depends on the biotic and abiotic environment.

In the current paper, we attempted to analyse genet demography and its environmental determinants for the clonal grass *Festuca rubra* in a mountain grassland. First, we estimated genet recruitment and size distributions, total genet numbers and mortalities from molecular data obtained by RAPD (random amplification of polymorphic DNA). We used a simulation approach to infer the total numbers of genets from the sample in order to express recruitment of genets in relative terms, and to determine whether recruitment equals mortality (i.e. whether the population is in equilibrium). Second, we examined how genet structure of *F. rubra* depends on neighbourhood species composition and on tussock dynamics. This was made possible by the existence of time series of fine-scale ramet counts of all species on the plots from which ramets were collected for genetic analysis (Herben *et al*. 1995). This enabled us to link the molecular marker data to the fine-scale spatial structure of the biotic environment, both currently and in the past, and to ask whether two ramets are more likely to be genetically identical (i) if they come from patches of *Festuca* ramets that differ in size or history (i.e. advancing or retreating patches), or (ii) if they come from neighbourhoods that differ in species composition.

Methods

STUDY SITE

The study site is located in a mountain grassland in the Krkonoše Mountains, in the northern part of the Czech Republic (Severka settlement, *c*. 3 km north-west of Pec pod Sněžkou, latitude 50°41′42″ N, longitude 15°42′25″ E, altitude approximately 1100 m). The whole area has a harsh climate; mean temperature in the warmest month (July) was 16.5 °C (1995), 12.3 °C (1996) and 13.2 °C (1997) at the nearby climatic station (Pec pod Sněžkou, *c*. 900 m a.s.l.). The study site has a long winter with thick snow cover lasting from November until the end of April. The studied grasslands are maintained by mowing. Since their establishment in about the 17th century they have reached a stable species composition

as a result of prolonged stable management (see also Krahulec *et al*. 1997). The traditional management of the meadows consisted of mowing once a year and manuring once every few years.

There are only four principal grasses in the plots: [*Anthoxanthum alpinum* Á. Löve et D. Löve, *Deschampsia flexuosa* (L.) Trin., *Festuca rubra* L. and *Nardus stricta* L., hereafter referred to as *Anthoxanthum*, *Deschampsia*, *Festuca* and *Nardus*], and a single common dicot, *Polygonum bistorta* L. Although the system is species-poor at the large scale, the species intermingle at a fine scale, forming superficially very homogeneous stands; the species density is 2–4 species 10 cm^{-2} and 6–10 species 2500 cm^{-2}. Structure is dominated by small clumps of all the four grass species, with *Nardus* and *Anthoxanthum* forming clumps several cm in diameter, and *Deschampsia* forming larger clumps (Herben *et al*. 1995). The structure is dynamic over time and temporal autocorrelation of all species disappears over approximately 6 years (Herben *et al*. 1995).

FIELD DATA COLLECTION

Four permanent plots (S1 to S4) of 50×50 cm were established at the site in 1984–85. Partial analyses of data from these plots have already been published (Herben *et al*. 1995 and references therein). The plots were marked by vertical plastic tubes sunk to a depth of 20 cm in the soil. A sampling frame was positioned by fitting steel rods into these plastic tubes; this allowed the frame to be re-located in the same positions every year (precision better than 0.5 cm). The number of ramets of each species within a grid of 15×15 3.3×3.3 cm cells over these plots was counted every year in mid July from 1984 until 1997. Plots were clipped to a height of 2.5 cm after recording; clipping simulated mowing and was performed to ensure that there was no succession in the plots. The clippings were collected, sorted into species and used to determine above-ground biomass of each species. The ramet counts were used to calculate biomass per cell by multiplying ramet count of each species by the mean ramet biomass of that species and by summing these values over all species.

As the reported study was part of a larger experimental study of grasslands, two plots (S1 and S3) were manured in autumn 1985, 1989 and 1993 (cow manure, following the traditional treatment on these plots) adding total N at 17 g m^{-2}, NO_3-N at 0.2 g m^{-2}, NH_4-N at 3.8 g m^{-2} and PO_4-P at 2.4 g m^{-2}.

GENET SAMPLING

In 1997, we sampled *Festuca* ramets from all four permanent plots for a RAPD analysis to identify individual clones. We proceeded from the lowermost left corner of each plot row-wise from left to right, determining the number of ramets to be sampled in each cell (1/12 of the total) from 1996 counts. Data from the previous year were used rather than disturbing the plots prior to genet sampling. This is valid because temporal autocorrelations are high over a time lag of 1 year (Moran's I = 0.5–0.8). The required numbers of ramets were taken from each cell at random. This procedure yielded 14–127 ramets per plot.

RAPD ANALYSES

All ramets collected were carefully washed and stored at room temperature after drying over silica gel. Total DNA was extracted according to the modified CTAB-method (Terauchi 1991; Štorchová *et al*. 2000). A modified version of the PCR-RAPD procedure of Williams *et al*. (1990) was used in order to identify genets. Amplification reactions were carried out with six primers, OPA-8, OPA-10, OPA-20, OPB-3, OPB-8 and OPB-11 (Operon Technologies Inc., Alameda, CA, USA) for each sampled shoot. Each 25-μL reaction volume contained 17.5 μL DDW, 2.5 μL 10× PCR buffer, 0.5 μL dNTP (2.5 mM each), 0.5 U *Taq* DNA polymerase (TaKaRa Ex taq, TaKaRa Biomedicals, Oohtu, Japan), 10 pmol of a primer (Operon Technologies Inc.), and 50 ng sampled DNA. Amplification was performed with a programmable thermal cycler (RoboCycler GRADIENT 96, Stratagene, La Jolla, CA, USA). Each reaction was passed through one cycle of 3 min at 94 °C, and 45 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C. Amplification products were analysed by electrophoresis in 2% agarose gels with a 0.5× Tris-borate-EDTA (TBE) buffer system including ethidium bromide, and 100 bp DNA ladder marker (New England Biolabs, inc., Beverly, MA, USA) was used to determine sizes of the amplified DNA products. Each run of each gel included a positive control that included the sampled DNA obtained from a single genet and a negative control that did not include any sampled DNA in order to confirm accuracy and reproducibility of the RAPD technique. Gel images were recorded by using a CCD camera system (Densitograph AE-6905C, ATTO, Tokyo, Japan) and a gel image of each sample was compared with all other images of the samples on a computer screen. The six selected primers gave 36 polymorphic bands, which were then used for genet identification; pairs of ramets that differed in band patterns were considered to belong to different genets. Each of the selected primers could distinguish DNA sampled from four different genets. We conducted the analyses using all of the six primers for all samples.

DATA ANALYSIS: PROBABILITY THAT TWO RAMETS BELONG TO THE SAME GENET, AND SEEDLING ESTABLISHMENT

To calculate the probability of seedling establishment, we used the approach of Harada *et al*. (1997), who showed that the probability that two ramets belong to the same genet over a distance r is $k(e^{-cr})/\sqrt{r}$, where k is a positive constant (corresponding to ramet size), $c = \sqrt{(2u/v\sigma^2)}$, where σ^2 is variance of distance between

mother and daughter ramet, v is the probability that a new ramet is formed by vegetative growth, and u is the probability that a newly formed ramet is of seedling origin. First, distances between all ramet pairs were calculated and the genetic identity of these ramets was determined. The actual number of vegetatively formed ramets was taken from the demographic data on *Festuca* from the same habitat (Herben *et al*. 1993). Under the assumption of population equilibrium, recruitment should equal mortality; because overall ramet annual mortality rate is approximately 0.3 yr^{-1} (Herben *et al*. 1993), the number of ramets formed can be calculated from the total ramet density m^{-2}. Mean distance between mother and daughter ramets in *Festuca* is 0.8 cm (R. Wildová, unpublished data). We fitted the formula by means of non-linear regression (Marquardt algorithm, SPSS 2000); we used a weighted form of the regression, where each point was assigned a weight proportional to the number of observations on which it was based. The regression coefficient was used to estimate the ratio of vegetatively and sexually formed ramets. The analyses were run for plots 1–3 separately. Plot 4 was not analysed because the number of ramets sampled there was low.

DATA ANALYSIS: ESTIMATION OF TOTAL GENET NUMBER

We used a simulation model to determine the sampling properties of the data set, and to infer genet sizes and total genet numbers from the sample. First, we generated many possible spatial arrangements of genets using a specified set of assumptions, and assigned all ramets in the plots to them. This represented the (unknown) underlying distribution of genets in the grassland. We then sampled ramets out of this set using exactly the same sampling procedure as used in the field. This established a relationship between a given underlying distribution of mean sizes and total numbers of genets (under the specified assumptions) and the sampled genet size and genet number. This was performed as follows:

1. In each plot, a given number of genet 'centres' were randomly distributed on a non-toroidal plane whose side was 2 × mean genet size + plot size, with the study plot in its centre. Each of these genets was assigned a size which was either constant for all the genets, or drawn from a gamma distribution with the same mean and CV of 0.3 and 0.9, respectively. The number of genets distributed in different runs was 5, 10, 20, 50, 100, 200 and 500. Mean genet sizes were 5, 10, 20, 30 and 50 cm. One hundred realizations of each parameter combination were run.

2. The spatial positions of all *Festuca* ramets in the plot were used to assign them to these genets. Each ramet was assigned to a given genet when the distance between the ramet and the centre of that genet was smaller than the size of that genet. If a ramet could have been assigned to more than one genet using this rule, one of the candidate genets was selected at random. If any ramet could not be assigned to any genet using this rule, the whole run was discarded. The whole combination of genet size and number was discarded and not used for later fitting if the percentage of successful runs was less than 10%.

3. Ramets with the genet identities assigned to them by simulation were sampled using exactly the same sampling procedure (i.e. sampling ratio and spatial pattern of sampling) as was used in the field, and the number of genets and their sizes were determined from this sample. Size was defined as the maximum distance between sampled ramets that belonged to the same genet. A 95% confidence interval was calculated for these statistics using 1000 bootstrap randomizations.

This procedure produced a distribution of sampled values for each combination of genet number and size underlying the 'true' numbers and sizes. This relationship was used to predict the underlying true genet sizes and numbers from the sampled values by fitting a cubic surface with true values as predicted variables, and sampled values as predictors (using the Marquardt algorithm, SPSS 2000). A separate surface was fitted for each plot; values from runs with the three different variation coefficients for genet size were pooled.

DATA ANALYSIS: GENET IDENTITY, NEIGHBOURHOOD SPECIES COMPOSITION AND HISTORY OF THE STAND

The following approach was used to link genet identity with the spatiotemporal information on the plots. First, all pairs of neighbouring ramets (defined as ramets in adjacent cells, i.e. with average distance between them of less than 1.41 × 3.3 cm = 4.7 cm) with known genet identity were selected. For one randomly selected ramet of this pair we determined species composition in its neighbourhood (number of ramets of *Festuca*, *Nardus*, *Deschampsia* and *Anthoxanthum*) in the year of sampling (1997) as well as the temporal change of *Festuca* density in its neighbourhood over the four preceding years (1993–96). Each of these variables was determined for three different sizes of neighbourhood around the ramet: only the 3.3 × 3.3 cm cell containing this ramet, the cell containing this ramet and its four-cell neighbourhood, and the cell containing this ramet and its eight-cell neighbourhood. Logistic regressions were then run to identify significant effects of these variables as predictors of the genetic identity of the ramet and its immediately neighbouring ramet. Plot (treated as a nominal variable) was used as a cofactor. The fffect of *Festuca* density in the year of sampling was fitted before densities of other species or *Festuca* density in the past were fitted separately and tested for significance. To determine whether the effects of these variables differed between plots, their interaction with plot was also fitted and tested. Analysis of deviance was used to test all the effects. The calculations were made using S-plus 2000 (MathSoft 2000).

Because this technique may use a cell more than once (if the ramet present in the cell has more than one

neighbouring ramet with known genetic identity), and thus inflate statistical significance, we also generated random subsets of the data with each randomly selected neighbourhood used only once. Twenty such subsets were generated and the logistic regression was run in the same way as described above.

Spatial and temporal autocorrelation of *Festuca* ramet density were calculated from the ramet counts in the plots using Moran's *I* (Upton & Fingleton 1985). Approximate significance of these correlations was determined by the full randomization of the grid data for the spatial correlations, and by randomization of the temporal sequence of individual grids for temporal autocorrelation.

Results

SPATIAL STRUCTURE AND DYNAMICS OF *F. RUBRA* RAMETS

There were 553 and 1183 ramets of *Festuca rubra* in plots 1 and 3, respectively; ramet numbers were considerably lower in the two remaining plots (248 in plot 2 and 107 in plot 4). *Festuca* ramet density showed significant autocorrelation across up to three 3.3×3.3 cm cells (plot 1, 1–3 cells; plot 2, 1–3 cells; plot 3, 1–2 cells; plot 4, 1–3 cells). All plots had rather similar spatial structure of *Festuca*, with plot 2 having slightly stronger aggregation at the small scale (Fig. 1). Temporal autocorrelation of *Festuca* showed stronger variation between plots, with plot 2 having the most stable clumps (range of significant correlations: plot 1, 1–3 cells; plot 2, 1 cell; plot 3, 1–2 cells; plot 4, 1 cell). In plots with low *Festuca* density (2 and 4) decrease of temporal autocorrelation was considerably weaker than that in plots with higher *Festuca* density (Fig. 1).

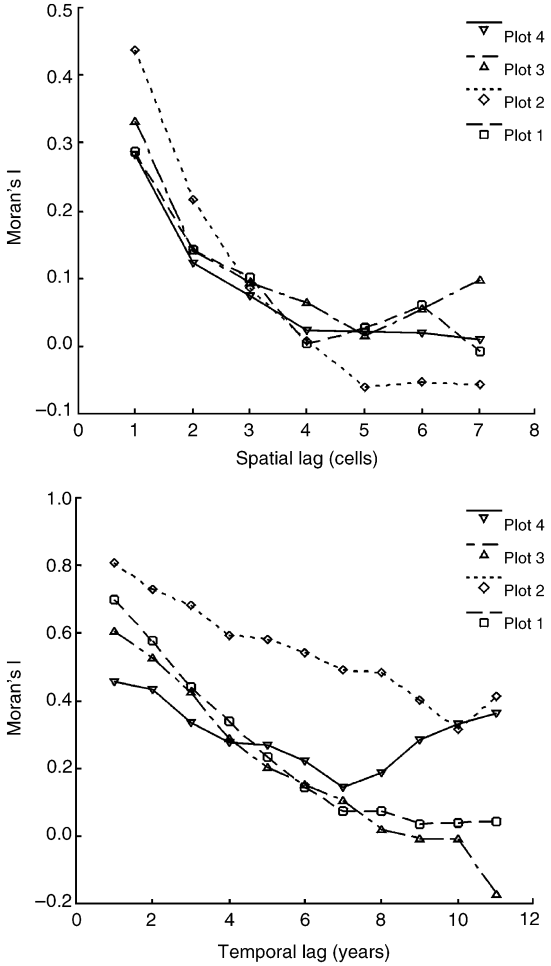

Fig. 1 Autocorrelograms of *Festuca rubra* ramets in space and time. One cell is 3.3×3.3 cm.

NUMBER OF GENETS

Out of a total of 224 ramets analysed, we found 145 different genets (Table 1). Most of the genets (98) were found only once; the genet with the highest number of ramets was sampled nine times. Therefore, the sampled ramet : genet ratio was very low, ranging from 1.40 to 1.65 depending on the plot. Mean genet size per plot (calculated as the distance between centres of cells with the most distant ramets belonging to one genet) was 1–2.5 cm; its distribution was highly skewed, with most genets having no estimate of size as they were found only once. The greatest observed distance between two genetically identical ramets was 6 cells (20 cm).

Coefficients of determination of cubic regressions of 'true' genet numbers and genet sizes against the sampled genet numbers and sizes were very high. The R^2 for prediction of genet numbers ranged between 0.85 and 0.92, depending on the plot; values for prediction of genet sizes were between 0.53 and 0.70. Using the parameters of the cubic polynomial, the numbers of genets found by genetic analysis of the plants ranged from 57.8 to 242.1 and genet sizes (maximum distance between genet centre and any ramet belonging to it) ranged from 3.4 to 8.5 cm (under the assumptions of the sampling simulation; Table 2). Because the total number of ramets per plot is known, the ramet : genet ratio in a plot can be calculated by dividing the total number of ramets by the total number of genets. This yields consistently higher values in plots with more *Festuca* ramets (1 and 3 vs. 2 and 4), and, except for plot 2, higher values than for the sampled ramets (Table 1).

PROBABILITY OF GENET IDENTITY, GENET RECRUITMENT AND MORTALITY

The frequency of pairs of sampled ramets being genetically identical was a decreasing function of distance between samples (Fig. 2; the low number of sampled points accounted for the low value at zero distance in plot 2). Fitting the formula of Harada *et al.* (1997) to this data set always yielded positive values of the coefficient *c* in the formula (negative values cannot be interpreted). The fit was high ($r^2 > 0.95$) in two plots, but lower in plot 2, where numbers of ramets sampled were very low (Table 3). This yields values of the ratio between

Table 1 Summary of genetic analyses of *Festuca rubra* ramets. Field sampling was done using an approximately constant sampling ratio; therefore, different numbers of ramets sampled in each plot reflect differences in the overall *Festuca* ramet density between the plots. Standard deviation of the ramet : genet ratio is based on a resampling procedure

	Ramets sampled	Number of genets found	Observed ramet : genet ratio		Observed mean genet size (cm)	
			Mean	SD	Mean	SD
Plot 1	54	37	1.46	0.77	2.51	4.97
Plot 2	29	21	1.38	1.12	1.55	4.05
Plot 3	127	77	1.65	1.23	2.13	4.20
Plot 4	14	10	1.40	0.70	0.94	1.99

Table 2 Mortality of genets calculated using the following assumptions: (i) all genets are of identical size in the plot (calculated by the sampling simulation), (ii) ramets counted in the field are divided between these genets, (iii) ramet mortality rate is independent (assumed to be 0.3 yr^{-1}) and (iv) death of a genet occurs when all its ramets die. Source: F, value estimated by field measurement; C, value calculated or estimated by sampling simulation

	Estimated total number of genets per plot	Estimated mean size (radius) of a genet (cm)	Number of ramets counted	Estimated ramet : genet ratio	Estimated genet *per capita* mortality per year	Genets dying per year per m^2
Source	C	C	F	C	C	C
Plot 1	143.9	8.1	737	5.1	0.002	1.2
Plot 2	241.1	8.5	299	1.2	0.225	216.6
Plot 3	242.1	4.9	1014	4.2	0.006	6.3
Plot 4	57.8	3.4	155	2.7	0.040	9.2

Table 3 Estimated long-term average seedling establishment rates using the formula proposed by Harada *et al.* (1997). Plot 4 is not included because of low number of genets

	No. of ramets sampled	R^2 non-linear regression	Regression coefficient *c*	Ratio of generative to vegetative ramets	Ramet density per 1 m^2	New vegetative ramets per year if population constant	New genets per m^2 per year
All plots	225	1.00	0.184	0.0108	2205	661.5	7.2
Plot 1	54	0.94	0.185	0.0110	2948	884.4	9.7
Plot 2	29	0.66	0.037	0.0004	1196	358.8	0.2
Plot 3	127	1.00	0.203	0.0132	4056	1216.8	16.0

numbers of ramets formed sexually and vegetatively ranging between 0.0004 and 0.013. If the population of ramets is assumed to be in a stable state, using the information on long-term average ramet mortality rate (0.3 yr^{-1}), vegetative recruitment can be estimated to range between 350 and 1300 ramets m^{-2}. This yields a long-term frequency of genet establishment (by successful seedling recruitment) ranging between 7 and 17 genets m^{-2} yr^{-1} per year (Table 3).

Genet mortality can be estimated from the ramet : genet ratio. If ramet mortality is known and probabilities of death of two ramets are assumed to be independent, the genet mortality can be calculated as the product of mortalities of all its ramets. Based on the information from plots 1 and 3, this yields values of genet mortality ranging between 1.2 and 217 genets m^{-2} yr^{-1}.

GENETIC IDENTITY AND DYNAMICS OF *FESTUCA* TUSSOCKS

Two ramets were more likely to be genetically identical if they came from a cell where *Festuca* density in the year of sampling was low (Fig. 3a), independent of the type of neighbourhood used. This relationship was weakly statistically significant; although it was highly significant when the whole data set was used, it was significant in only about one-quarter of the random data subsets used (Table 4). The effect was not significantly plot-specific when the whole data set was used, although it was stronger in plots 2 and 3, and mildly reversed in plot 1 (Fig. 4). By contrast, it was significant in 4–10 random subsets of the data, indicating that the difference between plots cannot be discounted.

Table 4 Effect of *Festuca* density and its temporal change on the probability of genetic identity (i.e. the probability that two nearby ramets are genetically identical). Tests were by logistic regression, with plots and ramet density in 1996 as covariates. Plot 4 was excluded because of a low number of observations. Effects of *Festuca* density in 1996, 1995 and 1994 were tested separately, with the same set of covariates (Plot and *Festuca* density in 1997). Festuca96, *Festuca* density in 1996; Festuca95, *Festuca* density in 1995, etc.; *P*, significance level from the analysis of the whole data set; 'Significant tests', number of results significant at $\alpha = 0.05$ (out of 20) when the analysis was made on subsets of data to remove spatial dependence (see Methods). NB. Significance tests in different neighbourhoods are not independent

		One cell only		Five-cell neighbourhood		Nine-cell neighbourhood	
	d.f.	*P*	Significant tests	*P*	Significant tests	*P*	Significant tests
Plot	2	0.638	1	0.398	1	0.398	1
Festuca97	1	0.012	0	0.000	4	0.001	5
Festuca96	1	0.000	9	0.000	11	0.001	2
Plot × Festuca97	3	0.237	10	0.241	6	0.297	4
Plot × Festuca96	3	0.051	3	0.132	1	0.093	2
Festuca95	1	0.466	1	0.770	1	0.861	0
Plot × Festuca95	2	0.754	0	0.469	0	0.413	0
Festuca94	1	0.199	0	0.911	0	0.691	0
Plot × Festuca94	2	0.760	1	0.829	0	0.993	1

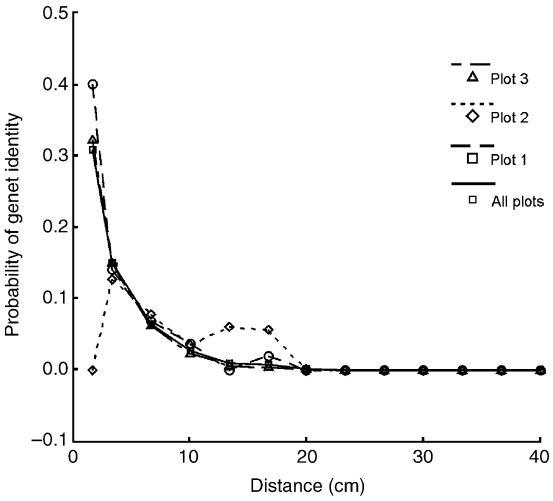

Fig. 2 Probability of genetic identity of ramets being the same, expressed as a function of ramet distance. Plot 4 is not included because of the low number of ramets sampled.

By contrast, there was a statistically highly significant (using both the whole data set and subsets; Table 4) effect of the temporal change of *Festuca* ramet density from 1996 to 1997 (year of sampling) with all three types of neighbourhood (just the cell itself, five-cell neighbourhood, nine-cell neighbourhood). A *Festuca* ramet in patches where *Festuca* density decreased from 1996 to 1997 had a lower likelihood of having a neighbour of the same genetic identity than a ramet in a patch with no decrease or a small decrease of *Festuca* ramet density (Fig. 3b; note there was an overall decrease in *Festuca* density from 1996 to 1997 and therefore the mean change from 1996 to 1997 is negative). The effect was consistent over plots except for plot 4, in which the number of ramets was very low; the interaction with plot was not significant.

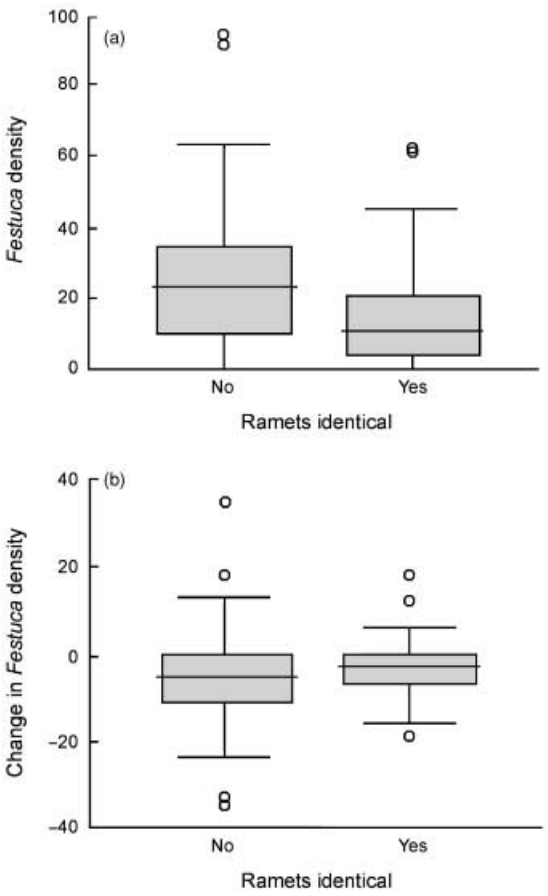

Fig. 3 Effect of *Festuca* density in the year of sampling (1997) (a) and the change of *Festuca* density from 1996 to 1997 (b) in the five-cell neighbourhood (the cell itself and the von Neumann neighbourhood) on the probability of two nearby *Festuca* ramets being genetically identical or not (all plots combined). Thick lines are medians, boxes indicate interquartile ranges of values, and lines connect the outermost value less than 1.5 times the interquartile range from the 25th or 75th percentile. Circles indicate other outlying values.

Table 5 Effect of density of other species in the year of sampling on the probability that two nearby ramets are genetically identical. Tests were by logistic regression, with plots and *Festuca* ramet density in 1996 as covariates. Effects of other species' densities were tested separately, with the same set of covariates (Plot and *Festuca* density in 1997). *P*, significance level from the analysis of the whole data set; 'Significant tests', number of results significant at $\alpha = 0.05$ (out of 20) when the analysis was made on subsets of data to remove spatial dependence (see Methods). Significant results are shown in bold type. NB. Significance tests in different neighbourhoods are not independent. Plot 4 was excluded from the analysis because of a low number of observations

		One cell only		Five-cell neighbourhood		Nine-cell neighbourhood	
	d.f.	*P*	Significant tests	*P*	Significant tests	*P*	Significant tests
Plot	2	0.638		0.398		0.398	
Festuca	1	0.012		0.000		0.001	
Deschampsia	1	0.582	0	0.483	0	0.306	0
Deschampsia × Plot	3	0.827	0	0.951	0	0.780	0
Nardus	1	0.039	0	0.017	0	0.012	0
Nardus × Plot	3	0.260	0	0.068	1	0.035	1
Anthoxanthum	1	0.173	0	0.003	5	0.000	4
Anthoxanthum × Plot	3	0.416	0	0.866	0	0.097	1

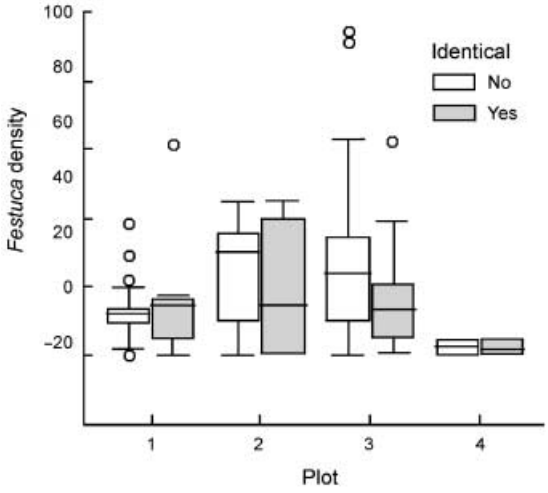

Fig. 4 Between-plot differences in the effect of *Festuca* density in 1997 in the five-cell neighbourhood on the probability of two nearby *Festuca* ramets being genetically identical or not. Thick lines are medians, boxes indicate interquartile ranges of values, and lines connect the outermost value less than 1.5 times the interquartile range from the 25th or 75th percentile. Circles indicate other outlying values.

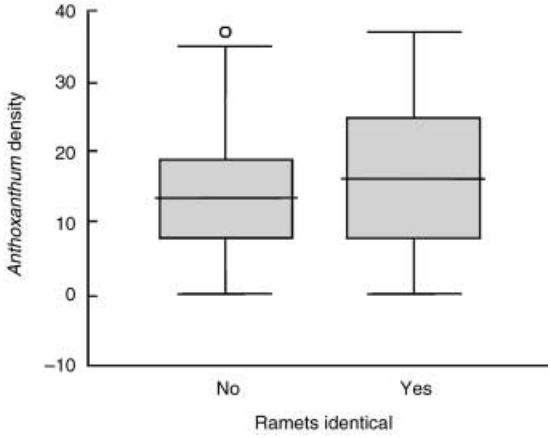

Fig. 5 Effect of density of *Anthoxanthum* in the five-cell neighbourhood in the year of sampling on the probability of two nearby *Festuca* ramets being genetically identical. Thick lines are medians, boxes indicate interquartile ranges of values, and lines connect the outermost value less than 1.5 times the interquartile range from the 25th or 75th percentile. The circle indicates an outlying value.

GENETICAL IDENTITY AND SPATIAL STRUCTURE OF THE GRASSLAND

Two ramets were more likely to be genetically identical when they came from a cell where density of *Anthoxanthum* was high in the year of sampling (Fig. 5). This was the case for all three types of neighbourhood used (Table 5). The effect is statistically highly significant when using the whole data set; in randomly subsampled datasets it remained significant in only about one-quarter of the datasets (Table 5). This effect is not due to a possible correlation of *Festuca* density with densities of other species in the cell because the linear effect of *Festuca* density was removed by the statistical analysis. By contrast, *Deschampsia* (the most common species in the grassland) had no similar effect (Table 5); the effect of *Nardus* was significant when the whole data set was used, but never significant in randomly subsampled plots. The species ranking in this effect is not the same as their correlation with *Festuca* density; Pearson correlation coefficients of *Festuca* density in cells with that of *Anthoxanthum*, *Deschampsia* and *Nardus* in 1996 were 0.053, −0.260 and −0.178, respectively.

Discussion

GENET POPULATION DYNAMICS

Total densities of genets in the current study are very high; the sampling simulation estimated that they range between 231 and 968 genets m^{-2} (Table 2). Similarly high numbers of genets are found in many other studies of clonal plants (Widén *et al*. 1994; Kudoh *et al*. 1999; Verburg *et al*. 2000). High genet numbers should lead

to low ramet : genet ratios and although the values reported in the above studies were larger than those found here, they are still only between 1.2 and 5.1. This may seem surprising for a plant with the capacity for clonal growth and genets therefore likely to consist of many ramets. However, many earlier studies reported only the number of genets sampled rather than the total number of genets; they therefore reported much lower numbers. In spite of the large number of studies on genet diversity in clonal plants, there are very few estimates of the total number of genets in populations. Fair *et al*. (1999) report rather similar values to ours for a prairie grass, *Bouteloua gracilis*, using an analysis of long-term mapping of the stands. The current estimates for *Festuca* seem to be in the ranges provided by other studies, e.g. those determined from mean recruitment rate relative to total ramet number (Suzuki *et al*. 1999), and from rhizome excavation results (Wildová 2004).

Recruitment estimation using the analytical approach of Harada *et al*. (1997) led to values in a similar range to that found in an earlier study at a larger scale (Suzuki *et al*. 1999), despite a different estimation technique. Whereas Suzuki *et al*. (1999) reported genet recruitment between 3 and 7 genets m^{-2} yr^{-1}, the numbers found here range between 0.2 and 16 genets m^{-2} yr^{-1} (although the lower value for plot 2 is less reliable because of the lower number of possible comparisons). This indicates that in spite of a small number of ramets per genet, genet turnover is not very high, with values between *c*. 0.1% and 1% per year (see also data on *Festuca rubra* by Harberd & Owen 1969). In addition, implant experiments conducted in the grassland (Herben *et al*. 2001) show that it is not unusual for a genet to survive for several years with only one or two ramets.

There is much less consistency in the mortality estimation. Estimates for three plots fall into a similar range to the estimates of recruitment (between 1.2 and 9.1 genets m^{-2} yr^{-1}); for plot 2, the plot with the lowest ramet : genet ratio, the estimate is unreasonably high at 216 genets m^{-2} yr^{-1}. If genet mortality is assumed to occur independently, the probability that a genet will die is an exponentially decreasing function of its ramet number; this yields very high mortalities for small genets (such as in plot 2) and very low mortalities for larger genets. Field observations show that ramet mortality is, to some degree, correlated as a result of local disturbance (Herben *et al*. 1993) and genet mortality is thus likely to be overestimated for small genets and underestimated for large genets, although the magnitude of the correlation (and thus of the bias) is not known. As genet mortality is underestimated when genets are large, the data do not provide strong evidence against the assumption that recruitment equals mortality and that the genet population is in equilibrium.

Estimates of both processes depend on the period over which they are determined. Recruitment is estimated as the long-term average, whereas mortality is estimated using the current values of the ramet : genet ratio. In reality, both recruitment and mortality are likely to vary between years, and year-to-year variation in genet recruitment is likely to be much larger than variation in genet mortality. Seedling establishment (i.e. genet recruitment) is sensitive to year-to-year variation in environmental conditions such as drought (Chesson 2000; H. Skálová, unpublished data), augmented by the strong temporal variation in seed input due both to irregular *Festuca* flowering between years (Herben *et al*. 1993) and to mowing, which in some years is too early for ripe seeds to be produced (H. Skálová, unpublished data).

GENET IDENTITY AND BIOTIC ENVIRONMENT AND HISTORY

The data collected show that many genets of *Festuca rubra* coexist in the grassland. Their size and structure is not independent of the spatial structure and history of the stand. Specifically, two ramets were more likely to be genetically identical if they came from a cell where the *Festuca* ramet density increased or remained the same over time. These findings indicate that local increases in *Festuca* density are usually the result of expansion of a single genet. This may be a result of fine-scale patches with favourable conditions in time and space, such as a very small disturbance or local decrease in density of other species allowing exploitation by a genet that is nearby at the appropriate moment. As favourable patches may appear at different places at different times on a fine scale, it is likely that different genets expand independently of each other. By contrast, patches where *Festuca* ramet density decreased were more likely to be composed of several genets. Such 'relict' ramets are remnants of earlier mixtures of several genets that have ceased to increase.

By contrast, the density or biomass of other species in the immediate neighbourhood had very little effect on the genetic identity of *Festuca* ramets occurring there and genets were distributed almost randomly through space. Most available data show that species identity makes little difference to competition if species do not differ in size (Keddy *et al*. 1998); however, in the case of *Festuca*, even local biomass differences turned out to be unimportant. This agrees well with results of a manipulation experiment in the same grassland that showed that fine-scale biomass variation had little influence on performance of implanted *Festuca* ramets (Herben *et al*. 2001). This is likely to result from low spatial variation in biomass, both above and below ground. Although any individual species in the grassland shows some degree of clumping, summed biomass shows little spatial aggregation, with autocorrelation occurring over only a rather short range (one 3.3-cm cell; Pecháčková *et al*. 1999; T. Herben, unpubl. data).

ISSUES IN THE CURRENT INTERPRETATION OF THE MARKER DATA

In most cases, interpretation of molecular marker data attempts to deduce dynamical processes from spatial

patterns at one point in time. This methodological issue has long been discussed in plant ecology (e.g. Goldberg 1995; Wilson 1995). In particular, commonly used approaches for recruitment estimation are based on the assumption that the current distribution of genet sizes and numbers is in equilibrium, i.e. that genet recruitment equals genet mortality (Harada *et al*. 1997; Kudoh *et al*. 1999; Jonsdóttir *et al*. 2000). If the system is in a transient state, the spatial arrangement of genets would reflect contingencies of germination and establishment, which would make dynamical interpretation of the observed patterns impossible. The assumption of equilibrium is difficult to assess as genet mortalities cannot be inferred from molecular data. In general, the almost complete lack of information on genet mortalities is a considerable drawback in studies on genet demography (De Kroon & Van Groenendael 1997; but see Fair *et al*. 1999); without more data on this process, no further progress in population biology at the genet level is possible.

The other important limitation is the lack of knowledge about total genet number (i.e. total population size). In our case, there was a clear difference between sampled and estimated ramet : genet ratio; the estimated value was greater than the sampled value, and even the ordering of the plots differed for sampled and estimated values. This highlights the importance of estimating total numbers of genets instead of relying on sampled numbers. In most herbaceous or grass species, the number of ramets in populations is too high for all ramets to be analysed. The information on total genet number must then be based on estimates only; however, the values obtained by sampling are by necessity less than the actual field values. As far as we know, a systematic approach to estimating total genet number in a population has not been previously published.

Conclusions

Despite high genet diversity, genet turnover is very low and genet life span is thus expected to be long. Behaviour of *F. rubra* genets was almost independent of fine-scale neighbourhood effects; we did not find evidence that these genets would specifically respond to the biotic microenvironments of the stand, or evidence of highly segregated genets that would indicate competition at the genet level. By contrast, *F. rubra* tussocks with divergent histories differ in the number of genets present. Ramet-level dynamics seem to dominate processes of genet dynamics, even in superficially homogeneous stands with many species intermingling at the ramet level. This may have important implications for our understanding of the mechanisms operating in grassland swards. Neutral processes at the ramet level would be more critical for genet dynamics than processes at the genet level. The ramet-level processes do not seem to respond strongly to the fine-scale heterogeneity owing to presence and abundance of other species. Genet-level selection processes are therefore likely to be tenuous, which may account for very large variation in functional traits such as tillering or vegetative growth in genet populations of grasses (Cheplick 1995; Skálová *et al*. 1997).

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