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Gene diversity in a fragmented population of *Briza media*: grassland continuity in a landscape context

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Summary

1 We investigated patterns of allozyme variation in demes of the grass *Briza media* in semi-natural grassland fragments within a mosaic agricultural landscape on the Baltic island of Öland. In the study area, *Briza* is both a characteristic species of old pastures and an early colonizer of young grasslands developing on previously forested or arable sites.

2 Generalized linear models revealed that descriptors of both present landscape structure and past grassland history are significant determinants of genetic variation in the *Briza* demes. Genetic structure and levels of within-deme diversity are influenced by the size of grassland fragments, the type of habitat surrounding the grasslands, the size/spatial extent of the demes, the geographic position of the demes and the historical continuity of the grassland fragments.

3 Gene diversity (H) was higher in demes from grassland polygons with a high proportion of adjacent grassland, higher in the more extensive demes, and decreased northwards within the study area.

4 The negative association between the inbreeding coefficient (F_{IS}) and grassland continuity is interpreted in terms of a two-stage colonization process: recruitment into young grasslands leads initially to spatial patchiness, but subsequent selection in maturing pastures occurs within an increasingly uniform and dense sward.

5 Despite a weak overall genetic structure (as indicated by Bayesian cluster analysis) the between-deme F_{ST} was significant. Linear discriminant analysis of within-deme allele frequencies grouped the demes according to the age and previous land-use history of their grassland polygons. The convergence of the allele frequency profiles in the younger grasslands towards those of the old grasslands is consistent with convergence of selective regimes as pastures mature towards an increasingly uniform, dense sward and characteristic species assemblage.

6 The genetic composition of demes of a grassland species appears to be influenced by the process of plant community convergence during grassland development – complementing the recent finding that convergence of species composition in experimental assemblages of grassland plants is dependent on the genotypic composition of the component species.

Key-words: allozymes, gene diversity, grassland history, habitat continuity, inbreeding coefficient, landscape mosaics

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Introduction

Changes in methods of agricultural production over the last century have involved the rationalization of land use and the widespread intensification of approaches to animal husbandry in Europe. Abandonment of traditional grassland management practices (mowing and grazing) has led to a massive reduction in the area of semi-natural grasslands (Bernes 1994; Eriksson *et al*. 2002; Poschlod & WallisDeVries 2002), and the distributions of many grassland plants have become increasingly fragmented and disjunct (e.g. Fischer & Stöcklin 1997). Semi-natural grasslands are characterized by high levels of plant species diversity, and the current interest in the sustainable management of biological diversity has focused attention on the status of the remaining grassland fragments. At the same time, fragmented grassland systems within the agricultural landscape provide a valuable arena for the study of the mechanisms – dispersal, migration, gene flow and adaptation – that determine levels of species diversity and gene diversity in natural populations.

There have been a number of recent studies of genetic variation in fragmented populations of grassland species (e.g. Young *et al*. 1999; Hooftman *et al*. 2004; Pluess & Stöcklin 2004; Van Rossum *et al*. 2004). The majority of these studies, in common with studies of fragmented plant populations in general, have focused mainly on stochastic processes and the roles of population size, density and isolation as determinants of levels of within-population gene diversity or the degree of interpopulation differentiation (e.g. van Treuren *et al*. 1991; Weidema *et al*. 2000; Hooftman *et al*. 2004; Vekemans & Hardy 2004). An increasing number of studies have also attempted to interpret the structure of genetic variation within and between fragmented plant populations in terms of metapopulation processes, and the dynamics of colonization and extinction in spatially discrete habitat patches (Cruzan 2001; Tero *et al*. 2003; Hilfiker *et al*. 2004). In contrast, the role of local adaptation and selection as determinants of genetic structure in fragmented population systems has received less attention (cf. Lönn *et al*. 1996; Kalisz *et al*. 2001; Volis *et al*. 2004).

A few studies (e.g. Giles & Goudet 1997; Cronberg 2002; Jacquemyn *et al*. 2004) have explored the possibility that, especially in long-lived perennial plant species, the structure of genetic variation may be associated with the historical properties of the landscape and with past population size rather than with present population size and isolation. The coastal archipelago along the northern Baltic coast, where a progression of new islands is being generated by ongoing isostatic uplift, has provided an unique system where genetic variation can be analysed in the context of the history of habitat availability (Giles & Goudet 1997; Cronberg 2002). However, for agricultural landscapes, it is more difficult to obtain temporal data on the distribution of habitats. Although aerial photographs provide data on

habitats and land use back to the first half of the 20th century, detailed maps that cover earlier historical periods are relatively rare. Studies of genetic variation have been carried out on fragmented populations of a woodland species, *Primula elatior* L., in Belgium where data on the distribution of forests in the agricultural landscape can be extracted from topographic maps that date back to the end of the 18th century (Jacquemyn *et al*. 2004). We have investigated genetic variation in a grassland species (*Briza media* L.) in a Swedish agricultural landscape that is covered by a series of aerial photographs and cadastral maps that extends back to the early 18th century.

Most studies of gene diversity in fragmented grassland plants have focused on the relict populations that remain after the severe reduction of previously wider distributions (e.g. van Treuren *et al*. 1991; Young *et al*. 1999; Van Rossum *et al*. 2004). *Briza media* is not, however, restricted to remnant fragments of grassland that have had a long historical continuity, but is also present in younger grasslands. This perennial grass is widespread and relatively common (Dixon 2002) and belongs to a suite of species that that are regarded as indicators of species-rich, unimproved, semi-natural grasslands in Sweden (e.g. Ekstam & Forshed 1992). In contrast to *B. media*, many of the other indicator species are known to be slow colonizers of new grassland sites and their occurrence is thought to be linked to a long history of grassland continuity (e.g. Eriksson 1998).

The study was carried out within a local agricultural landscape in which semi-natural grassland fragments are embedded in a mosaic of forest habitats and arable fields. All the present-day grassland fragments within the study area have been characterized in terms of their historical continuity (over the last 265 years) and their previous land use, as well as in terms of their present size and surrounding matrix of habitats. Within the study area, *B. media* is both widespread in grasslands that have had a long historical continuity and able to colonize grazed grasslands on abandoned arable fields after a few decades. Indeed, it has its highest frequencies in young grasslands on previously arable sites (M.T. Sykes, H.C. Prentice and O. Jonsson, unpublished data).

We investigate the extent to which gene diversity and the present structure of within-deme genetic variation in *B. media* can be explained by the historical continuity and past land use of grassland sites, and by properties of the present-day grasslands and their surrounding landscape matrix.

Materials and methods

THE STUDY AREA

The village of Jordtorp is located in the parish of Algutsrum on the Baltic island of Öland (Sweden). The study area (centred on 56°40′49″ N, 16°33′58″ E) covers 22.302 km² and includes the land associated with Jordtorp and four adjacent villages: Övetorp, Holmetorp/Hässleby,

Hult and Törnbotten. The limestone bedrock of the area is mostly overlain by neutral to weakly acidic morainic soils, and the present landscape consists of a small-scale mosaic of arable fields, semi-natural grasslands and different types of deciduous forest (cf. Forslund 2001).

GRASSLAND HISTORY AND CONTINUITY

Data on present and past land use and vegetation cover within the area are available from aerial photographs and historical maps (dating back to the early 18th century). Land-use/vegetation maps at the scale of 1 : 10 000 have been produced for the area for the periods 1728–33, 1801–10, 1821–51, 1938, 1959 and 1994/97 (M. Ihse, M. Kindström and H.C. Prentice, unpublished data). These maps reveal a progressive decline in the area of semi-natural grassland within the landscape over the last 300 years. Each of the grassland fragments (polygons) in the area has been characterized in terms of its history of grassland continuity. The land-use/continuity categories were obtained from a GIS overlay analysis, using the grassland polygons on the most recent land-use/vegetation map [derived from colour infra-red (CIR) aerial photographs from 1994 with field validation in 1997] as a base (M. Ihse, M. Kindström and H.C. Prentice, unpublished data).

Present-day grassland polygons that were classed as 'semi-natural grassland' on the 1994/97 base map can be assigned to seven categories of grassland continuity/land-use history, defined as years-before-1998 (O. Jonsson, H.C. Prentice, M.T. Sykes, M. Ihse and M. Kindström, unpublished data). The 'OLD' grasslands have had a continuous history of at least 265 years as grassland. The categories 'PF' (previously forest) and 'PA' (previously arable) represent intermediate-aged grasslands that were forested or used for arable cultivation at some stage during the last 177 years and which have subsequently had a grassland history of less than 177 years. Both the PF and the PA grassland polygons are divided into three subcategories based on the age of their transition from forest or arable to grassland. The PF_{100} and PA_{100} map polygons have been grassland since at least 1938 but were classed as forest or arable, respectively, in 1821/51 (*c.* 100 years of grassland continuity), PF_{50} and PA_{50} polygons have been grassland since at least 1959 but were classed as forest or arable in 1938 (*c.* 50 years of continuity) and PF_{25} and PA_{25} polygons (*c.* 25 years of grassland continuity) were classified as forest and arable in 1959. It takes approximately 15 years for abandoned arable fields to develop the signature of 'cultivated grassland' in CIR photographs, and a further 15 years to develop the signal of 'semi-natural grassland' (Ihse *et al.* 1993). The fact that the PA_{25} polygons were all recognizable as semi-natural grassland in the 1994 CIR aerial photographs indicates that arable cultivation ceased at least 15 years earlier. Thus (in 1998) these polygons must have had a grassland continuity that was longer than 19 years. By contrast, because much of the forest in the Jordtorp area is open and has a grass-

land ground-flora, the transition to semi-natural grassland after forest clearance may be more rapid than the arable-to-grassland transition. PF_{25} polygons that were interpreted as 'semi-natural grassland' in the 1994 CIR aerial photographs might thus have been cleared only 1 or 2 years earlier (giving a continuity as semi-natural grassland of less than 4 years in 1998).

Briza demes were sampled from grassland polygons belonging to six of the land-use/continuity categories: OLD, PF_{100}, PF_{25}, PA_{100}, PA_{50} and PA_{25}. A single *Briza* deme was sampled from a seventh grassland category ('RA' = recently arable) that was classed as 'cultivated grassland' (including both grass leys and recently abandoned arable fields) on the 1994 aerial photographs. In 1998, these grasslands were in the process of reverting to semi-natural grassland.

THE SPECIES: SITES AND SAMPLING

Briza media L. is a perennial, predominantly diploid (2*n* = 14) grass that has a wide native distribution in (predominantly) base-rich semi-natural grasslands in Europe (Dixon 2002). The species is a poor competitor (McLellan *et al.* 1997) but tolerates grazing, mowing, trampling and light shade (Dixon 2002). It has short rhizomes and forms loose tussocks that are seldom more than 10 cm in diameter. Vegetative recruitment has been reported under nutrient-poor conditions (McLellan *et al.* 1997). The seed bank is transient and germination occurs in the autumn (Thompson & Grime 1979). Reproductive studies suggest that *B. media* is predominantly outcrossing and has a low level of self-compatibility (references in Dixon 2002). The floral morphology shows adaptation to wind-pollination. An experimental study of caryopsis dispersal during a single season suggested restricted (predominantly < 1 m) local dispersal (Dixon 2002). However, longer distance dispersal by animals has also been observed (e.g. Fischer *et al.* 1996) and undehisced spikelets may be dispersed with hay (Dixon 2002).

Briza media is widespread within the Jordtorp grasslands. However, although *Briza* is a characteristic component of the species-rich grassland in the polygons that have had a long history of grassland continuity, it has its optima (in terms of both presence and frequency within 50 × 50-cm quadrats) in the younger grasslands on previously arable sites (M.T. Sykes, H.C. Prentice and O. Jonsson, unpublished data).

Vegetative shoots (ramets) were sampled from *B. media* demes within the Jordtorp area during July/August in 2001–02. The 48 sampled demes (Fig. 1) were chosen so that they represented large and small grassland fragments, and grassland fragments (polygons) with differing histories of habitat continuity. Samples representing the different grassland categories were spread throughout the study area (Fig. 1). Sampling proceeded outwards from the centre of each deme, following the rule that the sampled ramets should be separated from each other by, as near as possible, 5 m but not less than 3 m.
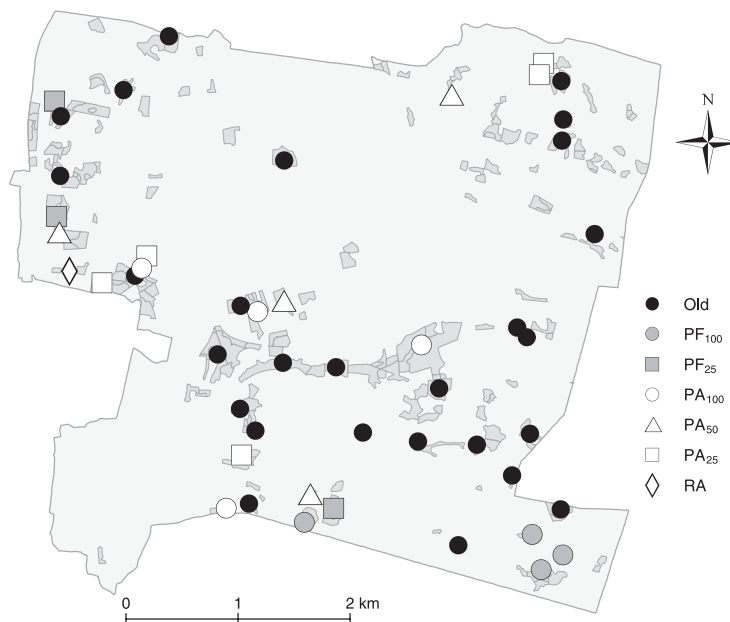
Fig. 1 Locations of the sampled *Briza media* demes within the Jordtorp area on the Baltic island of Öland. Map polygons classed as 'semi-natural grassland' are indicated by grey shading. The historical continuity classes of the grasslands from which the 48 demes were sampled are indicated by symbols. OLD = at least 265 years of grassland continuity; PA_{100}, PA_{50}, PA_{25} = grasslands on previously arable sites with continuity of *c.* 100, *c.* 50 and *c.* 25 years, respectively; PF_{100} and PF_{25} = grasslands on previously forested sites with continuity of *c.* 100 and *c.* 25 years, respectively; RA = recently arable.

This sampling procedure meant that the number of sampled ramets per deme was proportional to the spatial extent/size of the deme (with the maximum sample size limited to 26 ramets per deme). The sampled ramets were grown in pots in cold frames in the experimental garden in Lund.

ALLOZYME ELECTROPHORESIS

Allozyme electrophoresis was carried out on a total of 885 ramets. Leaf material (*c.* 1 cm from the base of a leaf) from the cultivated plants was extracted by grinding with washed sand in 5 µL of dithiothreitol extraction buffer (Tyler *et al.* 2002). The extracted material was taken up in 6 × 4 mm chromatography paper wicks and stored at −80 °C until electrophoresis could be carried out. Electrophoresis was carried out in horizontal 10.5% starch (Reppin 1000) gels using two buffer systems based, respectively, on lithium-borate/Tris-citrate (see Rosquist & Prentice 2000) and histidine-citrate (Wendel & Weeden 1990) buffers. Buffer system 1 was used to resolve the enzyme systems aspartate aminotransferase (AAT: E.C. 2.6.1.1), phosphoglucomutase (PGM: E.C. 5.4.2.2), phosphoglucose isomerase (PGI: E.C. 5.3.1.9) and triose-phosphate isomerase (TPI: E.C. 5.3.1.1). Buffer system 2 was used for isocitrate dehydrogenase (E.C. 1.1.1.42), malate dehydrogenase (MDH: E.C. 1.1.1.37), phosphogluconate dehydrogenase (PGD: E.C. 1.1.1.44) shikimate dehydrogenase (SKDH: E.C. 1.1.1.25) and uridine glucosepyrophosphyrolase (UGPP: E.C. 2.7.7.9). Staining protocols followed Wendel & Weeden (1990), with some modifications, and Manchenko (1994) for UGPP.

All nine enzyme systems showed variation that could be interpreted in terms of Mendelian alleles at one or more diploid loci. Data were scored for the following 11 polymorphic loci (numbers of alleles in parentheses): *Aat-1* (3), *Aat-2* (2), *Mdh-1* (2), *Skdh-1* (2), *Pgd-2* (5), *Pgi-2* (5), *Pgm-1* (4), *Tpi-1* (2), *Tpi-2* (3), *Ugpp-1* (3) and *Ugpp-2* (4).

STATISTICAL ANALYSES

Population structure

The Bayesian clustering program STRUCTURE (Pritchard *et al.* 2000) was used to investigate whether the sampled individuals could be assigned, on the basis of internal Hardy–Weinberg and linkage equilibria, to more than one population. The probabilites of division into 1–48 groups were calculated using 10 000 burn-ins and 10 000 repeats. We also used the program FSTAT (Goudet 2001) to calculate F_{ST} as a measure of the partitioning of genetic diversity between demes.

Linear discriminant analysis of history/previous land-use classes

Linear discriminant analysis (LDA) was used to test whether it was possible to discriminate between the seven grassland land-use history classes on the basis of allele frequencies in the sampled *Briza* demes, and to display the relationships between the demes. The *Briza* demes were used as within-group replicates, and the seven land-use history classes (OLD, PF_{100}, PF_{25}, PA_{100}, PA_{50}, PA_{25} and RA represented by, respectively, 27, 4, 3,

4, 4, 5 and 1 demes) as groups. A reassignment test was used to test the extent to which the individual *Briza* demes could be reassigned to the correct land-use history class. The LDA was carried out using the routine 'lda' in the library MASS (Venables & Ripley 2002) within the program package R 1.8.1 (R Development Core Team 2003).

Generalized linear models and model selection

Generalized linear models (McCullagh & Nelder 1989) were used to investigate the relationships between gene diversity and within-deme genetic structure in *B. media* and variables characterizing the extent of the demes and the present properties and historical continuity of the sampled grassland polygons. Three different response variables were used in the models: (i) within-deme gene diversity (H), corrected for sample size (Nei 1978) and assuming a pseudo-binomial model (McCullagh & Nelder 1989, p. 328); (ii) within-deme allelic richness [mean number of alleles per locus (Leberg 2002) at the polymorphic loci], assuming a gamma error distribution to allow for a linear relationship between sample size and allele richness that is not straight (see Leberg 2002); and (iii) the inbreeding coefficient (F_{IS}) within demes, estimated according to Nei (1987) and assuming a normal error distribution.

Minimal adequate models (Crawley 2002) explaining the variation in H, F_{IS} and allelic richness were constructed using the RSEARCH procedure in the program package Genstat, 6th edition (Lawes Agricultural Trust 2002).

We used the AIC (Akaike information criterion) to evaluate all possible models for a set of explanatory variables. A low AIC value indicates models that have a high likelihood and include a low number of variables (i.e. models that have a high predictive value). For each response variable we selected the set of models having the lowest AIC values (≤ 2 units from the lowest value; Burnham & Anderson 1998) for further evaluation. The AIC approach permits the inclusion of variables that may not be significant when subjected to standard significance testing. We therefore used the minimal adequate model approach (Crawley 2002) to obtain significance estimates for the individual explanatory variables in each of the selected AIC models. Only explanatory variables that caused a significant change in deviance when removed from a model (Crawley 2002) were retained in the final, minimal adequate, model. Several of the models with low AIC values contained no significant explanatory variables and were not considered further.

Thus, for each of the three response variables, we interpret those models that both have a low AIC and satisfy the requirements of a minimal adequate model [i.e. include only variables that are significant ($P < 0.05$)] when deviance ratios are compared with an *F*-distribution. Stepwise approaches typically result in a single explanatory model—meaning that models that have closely

similar explanatory power may be overlooked. The two-step approach allowed us to consider several models for each of the genetic response variables.

Interaction terms were examined for potential inclusion in all the final models that contained two or more explanatory variables. Plots of fitted vs. residual values were used to check all the final models for departures from expected error distributions. If two variables within the same model to a large extent explain the same variation in the response variable, the minimal adequate model method will retain only the variable that explains most of the variation. If the other variable explains a significant amount of variation that is not already explained by the first variable, both variables will be kept in the model. Highly correlated explanatory variables are likely to explain part of the same variation in the response variable and, consequently, only one of them will be retained in the minimal adequate model. When we present alternative models for a response variable, the explanatory variables in the different models may be correlated, but both models represent valid explanations, in their own right, of the variation in the response variable.

The explanatory variables in the models included descriptors of the geographic position and extent of the sampled *Briza* demes, the size of the grassland polygons from which demes were sampled, the adjacent habitat categories, the grassland history and the previous land use. Because *Briza* individuals were sampled according to the same spatial rules within each of the grassland polygons, the number of sampled individuals could be used as a descriptor of the size/extent (EXTENT: range 1–26, mean 19.2, SE 0.99) of the sampled demes. The geographic position of the *Briza* samples was characterized by distance (in km, starting from the origins) along the *x* (EAST) and *y* (NORTH) axes of the study area. The size of the sampled grassland polygons was characterized by the variables PERIMETER (units = m: range 171–2491, mean 539, SE 66) and AREA (units = m^2: range 1379–138 972, mean 13 901, SE 3121). The habitat/land use of the areas adjacent to the sampled grassland polygons was characterized by the percentage of the polygon perimeter (measured manually) bounded by each of the following land-use categories: GRASSLAND [including habitat interpreted as semi-natural grassland, 'alvar' grassland and 'hagmark' (forest-pasture) on the 1994 aerial photographs: range 0–78%, mean 31%, SE 3.8], ARABLE (including cultivated grasslands, grass leys and other, miscellaneous, grasslands: range 0–100%, mean 23.8%, SE 3.8) and FOREST (including closed forest and hazel thickets: range 0–100%, mean 33.6%, SE 4.7). Information on other types of adjacent land use was not included in the analyses. The grassland continuity (CONTINUITY) was characterized by the shortest time to the transition from forest or arable cultivation to semi-natural grassland, with the following time-classes: 265 years, 60 years, 39 years, 19 years, 4 years and 0 years. The land-use history of the grassland polygons was characterized by

the descriptor PREVIOUS LAND-USE, with the states 'arable' and 'forest' (grasslands belonging to the category OLD are assumed to have been originally derived from forest more than 265 years ago). Pairs of explanatory variables that have a Pearson correlation coefficient higher than 0.20 are: ARABLE–FOREST (−0.56), ARABLE–CONTINUITY (−0.23), ARABLE–NORTH (0.26), GRASSLAND–FOREST (−0.47), FOREST–EXTENT (−0.26), FOREST–EAST (0.39), AREA–PERIMETER (0.92), AREA–EXTENT (0.22), PERIMETER–EXTENT (0.29), CONTINUITY–EXTENT (−0.42), EAST–EXTENT (−0.41), CONTINUITY–EAST (0.21) and EAST–NORTH (−0.24).

Predictions from the models are based on the variable of interest while other variables in the model are kept at their mean values.

Results

ANALYSIS OF POPULATION STRUCTURE

The Bayesian cluster analysis indicated that all the *Briza* ramets from the study area can be regarded as belonging to the same genetic population. The probability for a one-population solution is close to unity, whereas the probabilities for larger numbers of populations are close to zero. The value for F_{ST} was 0.006, with a 95% confidence interval of 0.002–0.012 (bootstrapped over loci).

LINEAR DISCRIMINANT ANALYSIS OF GENETIC STRUCTURE

Figure 2 shows the first two axes of an LDA based on within-deme allele frequencies. The first two discriminant axes accounted, respectively, for 53.4% and 22.6% of the between-group variation in allele frequencies. The groups in the analysis were the seven continuity/previous land-use classes, with demes as within-group
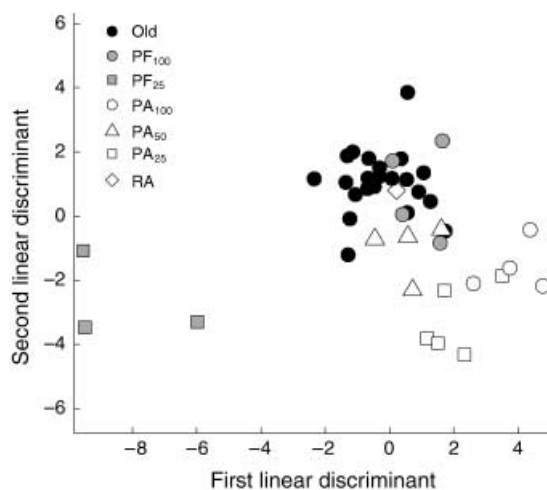

Fig. 2 Linear discriminant analysis of allozyme frequencies in 48 *Briza media* demes from the Jordtorp area on the Baltic island of Öland. The historical continuity categories for the grasslands from which the demes were sampled are indicated by symbols (see Fig. 1).

replicates. A reassignment test correctly reassigned all the demes classed as OLD, PF_{25}, PA_{100}, PA_{25} and RA to their correct groups. Only two demes were incorrectly reclassified (one PF_{100} and one PA_{50}, both reclassified as OLD). The OLD demes form a relatively tight group, with high scores on axis 2. Demes from grassland polygons that were previously forest but which reverted to grassland *c*. 100 years ago (PF_{100}) fall loosely within the OLD group of polygons, and the PF_{25} polygons form a distinct group with low scores on axes 1 and 2. The previously arable (PA) demes form a loose group with high scores on axis 1 and low scores on axis 2.

GENERALIZED LINEAR MODELS WITH GENE DIVERSITY AS THE RESPONSE VARIABLE

The mean, over loci, gene diversity within demes (*H*) ranged from 0.13 to 0.29, with a mean (over demes) of 0.23 (SE = 0.004).

The two best models for the response variable *H*, and their predictions, are shown in Table 1. Model 1 includes the significant explanatory variables EXTENT and GRASSLAND. Gene diversity is positively associated with the size/extent of the sampled demes and is also positively associated with the proportion of grassland habitats around the perimeter of the polygon. Model 2 includes the significant explanatory variables EXTENT and NORTH. As in model 1, there is a significant positive association between gene diversity and EXTENT. Gene diversity decreases northwards within the study area. Both possible interaction terms were tested for inclusion in the models but neither caused a significant decrease in deviance.

GENERALIZED LINEAR MODELS WITH THE INBREEDING COEFFICIENT AS THE RESPONSE VARIABLE

The within deme mean-over-loci inbreeding coefficient (F_{IS}) values ranged from −0.20 to 0.22, with a mean, over demes, of 0.02 (SE = 0.01).

The two best models for the response variable F_{IS} and their predictions are shown in Table 2. The explanatory variable CONTINUITY is significant in model 1, with a negative association between the inbreeding coefficient and grassland continuity. F_{IS} is positively associated with PERIMETER in model 1 and with AREA (polygon size) in model 2. The interaction term CONTINUITY × PERIMETER was tested for inclusion in model 1 but did not cause a significant decrease in deviance.

ALLELE RICHNESS AS THE RESPONSE VARIABLE

The mean number of alleles (per polymorphic locus) ranged from 1.2 to 2.3, with a mean over demes of 1.9 (SE = 0.03).

There was only one model for the response variable allele richness that included a significant explanatory

Table 1 Two models explaining gene diversity (Nei's *H*) in the *Briza media* demes. Summary predictions (calculated with the non-predicted variables kept at their mean values) and their standard errors (within parentheses) for a range of explanatory variable values are given for each model

	AIC	Term	d.f.	Mean deviance	Regression coeff. ($\times 10^3$)	*P*-value
Model 1	47.2	EXTENT	1	1.97	7.31	0.022
		GRASSLAND	1	1.60	1.67	0.039
		Error	45	0.35		
Model 2	47.3	EXTENT	1	2.50	8.04	0.012
		NORTH	1	1.54	−8.60	0.042
		Error	45	0.35		

	H
Predictions from model 1	
EXTENT (number)	
1	0.205 (0.010)
13	0.219 (0.005)
26	0.236 (0.005)
GRASSLAND (%)	
0	0.218 (0.005)
50	0.233 (0.004)
100	0.248 (0.011)
Predictions from model 2	
EXTENT (number)	
1	0.202 (0.010)
13	0.218 (0.005)
26	0.237 (0.005)
NORTH (km)	
0	0.240 (0.007)
2.5	0.226 (0.004)
5	0.213 (0.007)

Table 2 Two models explaining the inbreeding coefficient (F_{IS}) in the *Briza media* demes. Summary predictions (calculated with the non-predicted variables kept at their mean values) and their standard errors (within parentheses) for a range of explanatory variable values are given for each model

	AIC	Term	d.f.	Mean sum of squares	Regression coeff. ($\times 10^3$)	*P*-value
Model 1	47.8	CONTINUITY	1	0.035	−0.24	0.047
		PERIMETER	1	0.033	0.06	0.041
		Error	45	0.007		
Model 2	49.5	AREA	1	0.037	1.2×10^3	0.035
		Error	46	0.008		

	F_{IS}
Predictions from model 1	
CONTINUITY (years)	
0	0.054 (0.022)
4	0.054 (0.021)
19	0.050 (0.020)
39	0.046 (0.018)
60	0.041 (0.017)
265	−0.004 (0.016)
PERIMETER (m)	
171	−0.003 (0.016)
1160	0.054 (0.021)
2491	0.131 (0.054)
Predictions from model 2	
AREA (m^2)	
1379	0.002 (0.015)
68 796	0.090 (0.035)
138 972	0.181 (0.076)

variable. The model had an AIC of 45. The explanatory variable EXTENT (with 1 degree of freedom) gave a mean deviance ratio of 28 (giving $P < 0.001$ when compared to an F-distribution). Allele richness is positively associated with the size/extent of the sampled demes.

Discussion

According to Bayesian cluster analysis (which includes no prior information on deme membership) the *Briza* demes in the Jordtorp study area can be regarded as belonging to a single, genetically cohesive population. Our study nevertheless provides evidence that local landscape history and the local distribution of habitats have had an impact on the structuring of genetic variation in *Briza* within the Jordtorp area. The F_{ST} analysis (which includes a priori information on deme structure) reveals that a small (0.6%), but statistically significant, part of the total diversity is explained by between-polygon variation. When an additional level of supervision (a priori information on land-use history) is included, the LDA reveals a pattern of allele frequency variation that is explained by the land-use history of the grassland polygons from which the *Briza* demes were sampled. In addition, generalized linear models show that a number of different descriptors of landscape structure and grassland history are significant determinants of gene diversity and of the internal structure of genetic variation within demes. Several of the models for different genetic properties of the demes included more than one significant explanatory variable.

Genetic structure and levels of diversity within demes are influenced by the type of habitat that surrounds the grassland polygons containing the demes, the size of the grassland fragments, the size/spatial extent of the demes, the position of the grassland polygons within the study area and the historical continuity of the grassland fragments.

GEOGRAPHIC POSITION

The explanatory variable NORTH had a significant effect in model 2 for gene diversity, with H decreasing northwards (Table 1). The effect of geographic position may be explained by spatial pattern in the landscape – on a larger scale than the present study was designed to address. Comparison of maps of the distribution of grasslands in the Jordtorp area at different times during the last 300 years suggests that the patches of grassland within the forested areas in the north have had a longer history of fragmentation and isolation than the southern grasslands (L. Jönsson, H.C. Prentice, K. Hall, M. Kindström and M. Ihse, unpublished data). The northward decrease in values for H is consistent with the expectation that gene diversity is more likely to have been lost (and not subsequently replenished) in demes that have a longer history of isolation than in demes in habitat fragments that were connected to each other less than 50 years ago (cf. Wright 1942). In the

present study, the variables NORTH and EAST were included in all the models that were evaluated. Apart from the effect of NORTH in the second model for gene diversity, there were no significant effects of geographic position. The significant effects of variables that describe deme extent or properties of the grassland polygons in our models are therefore not directly confounded with the effects of geographic position (cf. Jacquemyn *et al.* 2004).

THE EXTENT OF THE DEMES AND THE SIZE OF THE GRASSLAND POLYGONS

The variable EXTENT was a significant predictor of H in both the models for gene diversity. EXTENT is an approximate descriptor of deme size (deme density and spatial extent). The values of H were highest in extensive (larger) demes (Table 1), in agreement with the general prediction that large populations will be less susceptible to genetic drift (cf. Wright 1942).

EXTENT was also a significant predictor of allelic richness. We adopted a sampling strategy that would allow valid comparisons of data on internal structure (F_{IS}) in different demes. However, the decision to use the same spatial sampling rule in all demes means that small (and/or spatially restricted) demes are represented by few individuals. Because the probability of detecting alleles – and thus the estimates of allelic richness – are strongly related to sample size (Sjögren & Wyöni 1994; Leberg 2002), the sampling design restricts the possibilities for the analysis of associations between allelic richness and deme size. As would be expected on the basis of the relationship between sample size and the probability of detecting alleles, there was a significant positive association between deme size/extent and the numbers of alleles in the demes.

The descriptors of polygon size, PERIMETER and AREA, were each significant in one of the models for F_{IS} (Table 2). Larger polygons showed higher F_{IS} values. The internal area and the perimeter properties of habitat fragments are predicted to influence both patterns of recruitment to demes and the internal diversity of microhabitats within the fragments.

SURROUNDING HABITAT

The proportion of grassland habitats surrounding the sampled polygons showed a significant positive association with gene diversity (H) in the *Briza* demes. The variables GRASSLAND, ARABLE and FOREST describe the landscape matrix in which the *Briza* demes are embedded. If there is a high proportion of surrounding grassland, it is probable that a *Briza* deme will be adjacent to other grassland fragments that contain *Briza* demes. The positive association between H and GRASSLAND is consistent with the expectation that proximity to multiple source-demes will enhance the variability of incoming gene flow (Slatkin & Voelm 1991; Lönn & Prentice 2002).

CONTINUITY

The seven land-use history categories include information on both grassland continuity and the type of habitat from which the grasslands with shorter continuity were derived. The LDA demonstrates that the previous land use and the historical continuity of the grasslands have both had an impact on allele frequencies within the *Briza* demes. Jacquemyn *et al.* (2004) also found an overall relationship between the frequencies of molecular markers and habitat age in the forest herb *Primula elatior*.

In the generalized linear models, the explanatory variable CONTINUITY (describing grassland history) was significantly negatively associated with the inbreeding coefficient (F_{IS}). *Briza* demes from grassland polygons with a long historical continuity are characterized by lower levels of internal structure (F_{IS}) than demes from polygons with younger grasslands (Table 2).

In a study of the colonization of forest fragments of differing ages, Verheyen *et al.* (2003) found that the frequencies of individual herb species in the fragments were a function of forest age and distance from species-specific colonization sources. There was also a relationship between the frequencies of the fastest colonizers and the environmental properties of the sites. Verheyen *et al.* (2003) interpret their results in terms of a 'two-stage' colonization process. The initial abundance pattern of species is determined by diaspore availability during the establishment phase. This phase is then followed by progressive environmental sorting of species with increasing age of habitat.

A comparable scenario can be envisaged for genetic variation in the *Briza* demes, with initial colonization by seed resulting in a spatial patchiness of genetic variation (Wahlund effect), reflecting local founder effects (cf. Lian *et al.* 2003; Proffitt *et al.* 2003), within the younger grassland polygons. McLellan *et al.* (1997) demonstrated that *Briza* is sensitive to competition from the surrounding vegetation and that it performed best in large experimental gaps. Directional selection [analogous to the 'environmental sorting' of Verheyen *et al.* (2003)] during the process of grassland development in the older polygons may have contributed to the loss of the patchy structure created by initial seed recruitment (Alvarez-Buylla *et al.* 1996). The homogenizing effects of internal gene flow by pollen may also be expected to lead to a successive reduction of internal spatial structure (F_{IS}) (Crow & Aoki 1984), especially if a decreasing availability of gaps for establishment also reduces the opportunities for external recruitment by seed in the closed sward of older grasslands (e.g. Rusch & Fernández-Palacios 1995).

The potential for local adaptation is well documented in grasses (Davies 1993). Studies of *Festuca ovina* in a fine-scale mosaic of grassland habitats on southern Öland indicate both fine-scale environmental sorting of genetic variation and an adaptive response to local

habitat manipulation (Prentice *et al.* 1995, 2000). In contrast to the situation further to the south on Öland (cf. Prentice *et al.* 1995), the old grasslands in the Jordtorp area are relatively homogeneous in terms of plant community composition (M.T. Sykes, H.C. Prentice and O. Jonsson, unpublished data). Directional selection (following an initial colonization phase), in an increasingly homogeneous habitat, may have contributed to the negative association between F_{IS} (internal genetic structure) in the *Briza* demes and grassland continuity. In a study of the demographic genetics of a pioneer tree species, *Cecropia obtusifolia*, Alvarez-Buylla *et al.* (1996) also suggest selection in a successively more uniform environment as a possible explanation for the loss of fine-scale genetic structure.

On the landscape scale, direct or indirect (with allozymes acting as markers for selected genes) selection appears to be implicated in the overall relationship between grassland history and allele frequency variation in *Briza*. Demes derived from the seven grassland history/continuity categories are grouped on the basis of their multilocus allele frequencies (regardless of their geographic origins) (cf. Figs 1 and 2). The convergence of the allele frequencies towards those in the OLD demes is interpreted as a response to the convergence of selective regimes during the succession from young grasslands, developing on previously forested or arable sites, towards species-rich grasslands that have an increasingly uniform and characteristic structure and composition (cf. Booth & Grime 2003). Booth & Grime's (2003) study of experimental assemblages of grassland species suggests that convergence of species composition in developing grasslands is facilitated if the component species are genetically diverse. The present study of *Briza* suggests, reciprocally, that the genetic composition of populations of a grassland species may be influenced by the process of plant community convergence during grassland development.

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Supplementary material

The following supplementary material is available online from www.Blackwell-Synergy.com

Table S1 Sampling data, gene diversity and F_{IS} values for the *B. media* demes.