

Contributed Paper

Effect of Rotational Shepherding on Demographic and Genetic Connectivity of Calcareous Grassland Plants

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Abstract: *Response to habitat fragmentation may not be generalized among species, in particular for plant communities with a variety of dispersal traits. Calcareous grasslands are one of the most species-rich habitats in Central Europe, but abandonment of traditional management has caused a dramatic decline of calcareous grassland species. In the Southern Franconian Alb in Germany, reintroduction of rotational shepherding in previously abandoned grasslands has restored species diversity, and it has been suggested that sheep support seed dispersal among grasslands. We tested the effect of rotational shepherding on demographic and genetic connectivity of calcareous grassland specialist plants and whether the response of plant populations to shepherding was limited to species dispersed by animals (zoochory). Specifically, we tested competing dispersal models and source and focal patch properties to explain landscape connectivity with patch-occupancy data of 31 species. We fitted the same connectivity models to patch occupancy and nuclear microsatellite data for the herb *Dianthus carthusianorum* (Carthusian pink). For 27 species, patch connectivity was explained by dispersal by rotational shepherding regardless of adaptations to zoochory, whereas population size (16% species) and patch area (0% species) of source patches were not important predictors of patch occupancy in most species. [Correction made after online publication, February 25, 2014: Population size and patch area percentages were mistakenly inverted, and have now been fixed.] Microsite diversity of focal patches significantly increased the model variance explained by patch occupancy in 90% of the species. For *D. carthusianorum*, patch connectivity through rotational shepherding explained both patch occupancy and population genetic diversity. Our results suggest shepherding provides dispersal for multiple plant species regardless of their dispersal adaptations and thus offers a useful approach to restore plant diversity in fragmented calcareous grasslands.*

Keywords: calcareous grasslands, connectivity, dispersal vectors, genetic diversity, Germany, patch occupancy, zoochory

Efectos del Pastoreo Rotacional sobre la Conectividad Genética y Demográfica de Plantas de Pastizales Calcáreos

Resumen: *La respuesta a la fragmentación del hábitat puede no estar generalizada entre las especies, en particular para las comunidades de plantas con una variación de métodos de dispersión. Los pastizales calcáreos son uno de los hábitats con mayor riqueza de especies en Europa Central, pero el abandono del manejo tradicional ha causado una disminución dramática de las especies en estos pastizales. En el Alb Franconio del Sur en Alemania, la reintroducción del pastoreo rotacional en pastizales previamente abandonados ha restaurado la diversidad de especies, y ha comenzado a sugerir que los borregos apoyan la dispersión de semillas entre los pastizales. Probamos los efectos del pastoreo rotacional sobre la conectividad genética y demográfica de las plantas especialistas de los pastizales calcáreos y si la respuesta de las poblaciones de plantas al pastoreo estaba limitada a especies dispersadas por animales (zoocoria). En específico, probamos modelos de competencia de dispersión y propiedades de fragmento focales y de origen para explicar la conectividad de paisaje con la información de ocupación de fragmento de 31 especies. Ajustamos los mismos modelos de conectividad a la ocupación de fragmento y a la información de microsatélites nucleares para la hierba *Dianthus carthusianorum*. Para 27 especies, la conectividad de fragmento se explicó por la dispersión*

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Paper submitted July 27, 2012; revised manuscript accepted July 5, 2013.

por pastoreo rotacional sin importar las adaptaciones a la zoocoria, mientras que el tamaño de población (16% de especies) y el área de fragmento (0% de especies) de fragmentos de origen no fueron importantes para predecir la ocupación de fragmento de la mayoría de las especies. [Correction made after online publication, February 25, 2014: Population size and patch area percentages were mistakenly inverted, and have now been fixed.] *La diversidad de micrositios de los fragmentos focales incrementó significativamente el modelo de varianza explicado por la ocupación de fragmento en el 90% de las especies. Para *D. carthusianorum*, la conectividad de fragmento por medio del pastoreo rotacional explicó tanto la ocupación de fragmento como la diversidad de genética de poblaciones. Nuestros resultados sugieren que el pastoreo proporciona dispersión para muchas especies de plantas sin importar sus adaptaciones a la dispersión y por lo tanto ofrece un acercamiento útil para restaurar la diversidad de plantas en pastizales calcáreos fragmentados.*

Palabras Clave: Alemania, conectividad, diversidad genética, ocupación de fragmentos, pastizales calcáreos, vectores de dispersión, zoocoria

Introduction

Habitat loss and fragmentation change the former widespread distribution of plants into disconnected patches varying in size and spatial isolation and threaten biodiversity because small and isolated plant communities are more prone to species loss and erosion of genetic diversity (Ellstrand & Elam 1993). For plants, population connectivity occurs through the movement of seeds and pollen. Seed dispersal contributes to demographic and genetic connectivity because it affects population growth rates, gene flow, and species persistence (Nathan & Muller-Landau 2000; Levin et al. 2003). Limited seed dispersal may lead to extirpation of small populations and prevent habitat recolonization (Cain et al. 2000; Piessens et al. 2005). Moreover, reduced connectivity increases genetic differentiation between populations and can reduce genetic diversity through drift and inbreeding if populations are small (Aguilar et al. 2008).

Habitat fragmentation affects both demographic and genetic processes and hence it is crucial to integrate ecological and genetic data in plant conservation research (Oostermeijer et al. 2003). The amplification of nuclear markers is a common approach to evaluate genetic diversity across populations varying in size and spatial isolation (Ouborg et al. 1999). Observational studies show that larger and well-connected populations exhibit higher genetic diversity than small, isolated populations (Honnay et al. 2007; Neel 2008). Computer simulations show that reduced connectivity can create genetic bottlenecks even in the absence of a sudden decrease in population size (Broquet et al. 2012). In plants inferences of gene flow from nuclear markers are affected by both seeds and pollen, but genetic connectivity may largely depend either on pollen or seeds, depending upon their interaction with vectors and the matrix (Petit et al. 2005). Pollen often travels longer distances than seeds, in particular for plants without seed dispersal adaptations. However, directed dispersal by animals can transport seeds in considerable numbers over large distances (Howe & Smallwood 1982). In this case, seed deposition in habitat areas can be substantial, but it is questionable whether

this effect occurs in species that are not dispersed by animals (zoochory).

Calcareous grasslands in Central Europe are dry, nutrient-poor, seminatural communities, which in many regions have been used traditionally for rotational shepherding, and are one of the most species-rich communities in Central Europe (Willems 1990). During the 20th century, cessation of shepherding and subsequent shrub encroachment led to a loss of up to 90% of calcareous grasslands and a dramatic species decline in remnant fragments (Poschlod & WallisDeVries 2002; WallisDeVries et al. 2002). Calcareous grasslands are important refugia for many species within an intensively used agricultural landscape.

Rotational shepherding may act as the primary driver of connectivity in remnant fragments of calcareous grasslands. A field experiment showed that seeds without dispersal adaptations are transported in the fleece of sheep, though in smaller numbers than seeds with adhesive appendages (Fischer et al. 1996). Also, most calcareous grassland forbs have a release height below vegetation height, which may reduce their potential for wind dispersal and thus reinforce the importance of animals as vectors (Diacon-Bolli et al. 2012). In the Franconian Alb in Germany, restoration of calcareous grasslands by rotational shepherding significantly increased species richness of specialist plants in grazed restored patches relative to patches that were ungrazed (Wagner et al. 2013). In the same plant community, Rico et al. (2012) tested alternative connectivity models and found that community-level colonization rates quantified by aggregate data of 48 habitat specialists was related to connectivity along shepherding routes. Habitat area of source patches, which often is equated with plant abundance, had no effect, but the diversity of microsites in focal patches, related to gap creation and establishment, contributed an increase in colonization (Rico et al. 2012).

The effect of landscape structure on the movement of seeds and pollen may largely depend on the interaction between vectors and dispersal traits (Maurer et al. 2003; Soons et al. 2004); hence, the response to habitat fragmentation cannot be generalized among species.

Calcareous grassland plants present a wide range of morphological dispersal traits, so a connectivity assessment on the basis of aggregate species data may be driven largely by zoochorous species and may not appropriately describe demographic or genetic connectivity for all species. We aimed to assess whether the effect of rotational shepherding on landscape connectivity of calcareous grassland specialist plants depends on dispersal-related traits. We also evaluated whether patch area or population size in source patches and the diversity of microsites in focal patches helped explain species patch occupancy. For *Dianthus carthusianorum*, a representative herb of calcareous grasslands in the study area and that is not zoochorous, we determined whether genetic connectivity was consistent with demographic connectivity. We applied the competing models of connectivity of Rico et al. (2012) with all individual species of the same plant community with sufficient data for statistical analyses and with nuclear microsatellite data for *D. carthusianorum*.

Methods

Study Area and Grazing Treatments

The study area of approximately 10×15 km in the Southern Franconia Alb in Bavaria, Germany, included 96 calcareous grasslands embedded within a matrix of agricultural fields, forests, meadows, roads, and settlements. Cessation of traditional shepherding and intensification of agriculture caused a rapid decline of calcareous grasslands of the Gentiano-Koelerietum pyramidatae vegetation (Oberdorfer 1978) from 970 ha in 1830 to 302 ha by 1989 (Dolek & Geyer 2002). In 1989, a restoration project was initiated to reconnect high-quality patches that were grazed and typically harbored most of the specialist plants (core areas) with patches abandoned since at least 1960 (restored patches) (Boehmer et al. 1990). In each of the 3 nonoverlapping shepherding systems, flocks of 400–800 ewes are herded in both directions along predefined routes. Core areas (0.05–10 ha, $n = 34$) and restored patches (0.004–1.4 ha, $n = 62$) are intermixed within each herding system, with 5–500 m between neighboring patches. Before restoration by shepherding, species richness of specialist plants in subsequently restored patches varied significantly as a function of patch area but not isolation (Wagner et al. 2013).

We used information from management records 1989–2009 to categorize the grazing treatment of all 62 restored patches: consistently grazed patches ($n = 26$) that since 1989 were grazed 3–5 times/year throughout the growing season, typically for less than 1 day each time; intermittently grazed patches ($n = 13$) that were grazed for a few years or at the end of the growing season; and ungrazed patches ($n = 23$) that were included in any herding system. Core areas were never abandoned and were grazed in the same manner as consistently grazed patches.

Species Data

We analyzed patch-occupancy data from a 2009 survey of 48 habitat specialist plants in all 96 calcareous grasslands, including core areas and restored patches, to assess the interaction between species dispersal traits and the effects of rotational shepherding and patch characteristics on species landscape connectivity. Surveys were carried out during summer 2008 and spring and fall 2009. Reliability of species detectability even in the absence of reproductive structures was corroborated by comparing results of independent surveys of different observers. Most perennial calcareous grasslands plants produce short-lived seeds (Poschlod et al. 2003), and species recovery from the seed bank after extinction is low (Poschlod et al. 1998; Zobel et al. 2000). Sensitivity analysis suggested that patch species richness and patch colonization rates are robust to variation in species detectability and seed bank longevity (Rico et al. 2012; Wagner et al. 2013).

Population size was quantified using 4 ordinal classes: 1, 1–3 individuals; 2, 4–39 individuals; 3, 40–99 individuals, and 4, ≥ 100 individuals. Patch area was digitized from orthophotos in ArcGis 9.3. To account for postdispersal effects, microsite diversity in focal patches was quantified by counting the number of dynamic structural elements likely providing regeneration niches on an ongoing basis. These elements were rock debris, identified by the presence of prominent fragments of calcareous rock; anthills; small mammal burrows; and erosion (small portions of loose soil). Relative change of species richness 1989–2009 in restored patches was significantly associated with this measure of microsite diversity, which indicates processes that promote gap creation, but was not associated with type of accompanying vegetation, which reflects differences in site conditions such as shading, moisture, and nutrient levels (Wagner et al. 2013).

Species Traits

We classified species by dispersal-related traits: release height, seed length, seed width, and dispersal by animals or wind (Table 1). There were no significant associations among traits (Pearson linear correlation for quantitative traits and point-biserial correlation between quantitative and binary variables); thus, we included all traits in subsequent analyses.

Parameterization of Connectivity Models

We used the connectivity approach of Rico et al. (2012) that parameterized a connectivity S_i index (Hanski 1994) with alternative dispersal models and source patch effects. The S_i index quantifies the connectivity of a focal patch i as $S_i = \sum_{j \neq i} \exp(-\alpha d_{ij}) A_j \cdot p_j$, where α is a constant scaling parameter, d_{ij} quantifies the distance

Table 1. Description of morphological traits related to seed dispersal.^a

Dispersal trait	Value	Sample size
Seed length	continuous (mm)	30
Seed width	continuous (mm)	30
Wind dispersal	1: present	8
	0: absent	22
Animal dispersal	1: present	13
	0: absent	18
Release height class	1: max < 30 cm	8
	2: min < 30 and max ≥ 30 cm	6
	3: max & min ≥ 30 cm	17

^aSource for the first 4 traits, Klotz et al. (2002). Source for release height class, Kleyer et al. (2008).

between a focal patch i and a source patch j ($i \neq j$), A_j is area of the source patch j , and p_j is species occupancy of the source patch j (absent, 0; present, 1).

The parameter d_{ij} was quantified with 5 models that varied in assumptions on species dispersal (Rico et al. 2012). In the geographic distance model (Eu), seeds are dispersed by a simple diffusion without matrix effects (d_{ij} = shortest interpatch Euclidean distance in kilometers). In the matrix resistance model (matrix), seeds can be intercepted by the intervening forest (d_{ij} = distance in kilometers across forest, measured in a straight line between patch i and patch j). The consistently grazed model (GrC), includes only core areas and restored patches grazed 3–5 times throughout the season, sheep are the main dispersal vector, grazing occurs consistently for seeds to be dispersed, and dispersal mostly occurs to neighboring patches (d_{ij} = number of patches that sheep traverse along the shepherding route from patch i to patch j ; high value of d_{ij} , 100, was assigned between patches unconnected by shepherding). The intermittently or consistently grazed (GrI) model is similar to GrC, but intermittent grazing is sufficient for dispersal; thus, all grazed patches are included. The grazed within the same grazing system (GrWS) model, differs from the previous 2 models because it does not include a distance effect; thus, seeds are equally likely to be transported anywhere within the same system (d_{ij} = 0 if in the same system, d_{ij} = 1 otherwise).

For each distance parameter d_{ij} , we tested 3 source patch effects. Model 1 included parameter p_j , all occupied patches are equal sources of seeds. Model 2 included patch area ($A_j \cdot p_j$) and the relationship between area and population size is positive. In addition to the variables evaluated by Rico et al. (2012), we included a third model that replaced A_j for population size n_j (ordinal categories: 1, 2, 3, 4). We tested additional transformations with interval midpoints for population size (2, 20, 70, and 150) to assess whether our classification (1–4) affected logistic

regressions. We did not observe differences in overall trends and thus present the results with categories 1–4.

The constant α is a species-specific dispersal constant that was optimized for each species by varying α from 0.1 to 10 in increments of 0.1. Because α is a constant-scaling d_{ij} , α was optimized for each connectivity model using the S_i with the parameter p_j . The α value with the lowest logistic regression deviance was selected for parameterizing each S_i (Supporting Information).

Species Occupancy Analyses

Analyses were performed for 31 of the 48 species with sufficient data (species occurrence of 15–85%). We used multimodel inference with the function dredge in the R-library MuMIn (Burnham & Anderson 2002) to rank and summarize each of the 15 S_i models. Akaike model weights w_m were summed for each parameter over all candidate S_i models containing the respective parameter to determine its relative importance. We applied multiple logistic regressions of the best S_i index and microsite diversity in focal patches to evaluate postdispersal effects on patch occupancy. Subsequently, we applied variation partitioning to determine the unique contributions of the 2 predictors. To assess sensitivity of results to variation in grazing treatment, we repeated the above analysis with only consistently grazed patches as focal patches.

Species Trait Analyses

We performed a chi-square test to assess significant differences in the proportion of the best-ranked connectivity models by species grouped by dispersal syndrome. In addition, we used the higher of the 2 species-specific logistic R^2 of the S_i shepherding models with a distance effect (GrC and GrI) as a response variable in multiple linear regression to assess whether strength of the response to shepherding connectivity is related to dispersal traits. We applied stepwise model selection with AIC to rank and select the best full linear regression model. All analyses were carried out in R (R Development Core Team, 2013).

Genetic Sample Collection and Microsatellite Analyses

We selected *D. carthusianorum* for genetic analysis because it occurs in many patches, but not at in high densities, and is easily detected throughout the growing season. *D. carthusianorum* has no persistent seed bank and no specialized dispersal adaptations (Klotz et al. 2002). In 2009, we collected leaf samples from 64 patches (hereafter populations): 31 restored patches, 25 core areas, and 8 additional sites where the species occurred in grass verges along roads or forest edges. We collected leaf tissue for all plants in patches with <40 individuals, whereas only 30–40 plants were sampled in

patches with >40 individuals. Leaf tissue was immediately dried in silica.

Genomic DNA was extracted with the DNeasy 96 Plant kit (QIAGEN). We amplified 15 microsatellite loci developed for related *Dianthus* species (MS-DINMADSBOX, MS-DCDIA30, MS-DCAMCRBSY, MS-DINCARACC, DCA 221, DCD 224, DCB140 [Smulders et al. 2000]; DCB109 [Smulders et al. 2003]; CB018a, CB057a; CB004a, CB027a, CF003a, CB011a, CB020a [Kimura et al. 2009]). Microsatellite amplifications were done using the QIAGEN Multiplex kit as follows: 0.2–0.4 μL (5 μM) of each primer, 4.7 μL of Master-mix, and 5–10 ng of genomic DNA in a reaction of 10 μL . PCR conditions followed those in Smulders et al. (2003). Approximately, 20% of samples were amplified twice to account for reliability, which was <2% of error. Fluorescent labeled PCR products were run on an ABI 3730X Automated Sequencer (Life Technologies, Grand Island, NY, USA) with 500 LIZ size standard. Electropherograms were analyzed using GENMARKER 1.91, and all genotypes were checked visually at least twice. Null alleles, allelic drop out, and genotyping errors were evaluated with MICROCHECKER (van Oosterhout et al. 2004). We only detected a high proportion of nonamplifications at 3 loci (DCA221, DCD224, DCD140), which were discarded from the data set.

Genetic Data Analyses

Departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for each population were tested using probability tests with 10000 Markov chain iterations in GENEPOP 3.4 software. Locus DC020a significantly deviated from HWE in 50 populations and was discarded from the data set. Some locus combinations showed statistically significant LD in some populations, but there were no recurrent patterns across locus pairs. Our final genetic set included 1613 individuals genotyped for eleven polymorphic microsatellite loci. In total, we had 2.2% of missing data for samples failing to amplify one or 2 loci. Population genetic diversity estimates such as allelic richness (A_r), observed (H_o), and expected heterozygosity (H_e) (Supporting Information) were estimated using GENALEX software. We corrected for the effect of sample size in allelic richness through rarefaction in HP-RARE (Kalinowski 2005).

We used the rarefied measure of mean allelic richness (A_r) because it was the genetic diversity estimate that produced the best model fit (homoscedasticity and residual distribution) and the highest R^2 in linear regression analyses. Patches with <4 individuals were excluded from statistical analysis ($n = 6$). We applied multimodel inference to rank each S_i parameter on genetic diversity. We performed significance tests and residual analysis for the best S_i index. Also, we evaluated whether ge-

netic diversity differed by population size and patch type (core areas, restored patches: consistently grazed, intermittently grazed, or ungrazed) with a type-II ANOVA followed by pairwise comparisons (TukeyHSD, familywise $\alpha = 0.05$).

Results

Connectivity Effects on Patch Occupancy and Dispersal Traits

Almost all species had significant positive regression coefficients for their best fitting S_i connectivity models (Table 2). Connectivity models of dispersal by sheep (GrC or GrI) were the best-ranked models for 27 of the 31 species. Specifically, GrI was the most supported model for 17 species, and the GrC model was the best-ranked model for 10 species. For 9 out of the 31 species, patch occupancy (p_j) was the best supported source patch parameter, population size (n_j) was the best parameter for 5 species, and patch area (A_j) was not the best parameter for any species. For 15 species the selected source patch parameter was not conclusive (relative importance <0.6) (Table 2). When restricting focal patches to consistently grazed patches, there was support for a distance-dependent effect of shepherding models (GrC or GrI), as there was with the full data set (Supporting Information). Although several species showed a change in top ranked model between GrC and GrI in either direction, *Pbleum phleoides* switched from the GrI to matrix resistance model, and *Euphorbia verrucosa* and *Hippocrepis comosa* from switched from geographic distance or matrix resistance to the GrC model (Supporting Information).

Postdispersal effects in terms of microsite diversity of focal patches significantly increased the model variance explained in most species, resulting in logistic R^2 of 0.1 (*Euphorbia cyparissias*) to 0.32 (*Trifolium montanum* and *Ononis spinosa*). Three species (*Salvia pratensis*, *Ajuga genevensis*, and *P. phleoides*) showed a nonsignificant effect. Variation partitioning suggested S_i connectivity alone had a larger contribution than microsite diversity for 21 of the 31 species (Table 2).

There were no significant differences in the proportion of the best-ranked models for species dispersed by animals or wind or species without dispersal adaptations ($\chi^2 = 7.99$, $df = 6$, $P = 0.24$; Fig. 1). Stepwise model selection included zoochory and seed length as the best linear regression model to explain the strength of association between patch-occupancy and shepherding connectivity. This model was significant with $R^2_{\text{adj}} = 0.15$ (AIC = -169.9; $df = 2$ and 27, $F = 3.5$, $P = 0.04$) and unbiased residuals without influential outliers. However, only zoochory was statistically significant ($P = 0.02$; seed length $P = 0.2$).

Table 2. Best performing parameters of the S_i patch connectivity index as ranked by the sum of Akaike model weights and relative contribution of patch connectivity (S_i) and microsite diversity (Elem) to the total variance explained by final models of patch occupancy for 31 habitat specialist plants of calcareous grasslands in the Southern Franconian Alb, Germany.

Species	Dispersal syndrome	n^a	Distance ^b	Best S_i index ^c			Final model ^d		
				Source	S_i z_coef	Logistic coefficient	S_i	Elem	Logistic coefficient
<i>Sanguisorba minor</i>	animal	79	GrI (0.98)	p_j (0.55)	1.394	0.20***	0.05	0.15	0.30*
<i>Arabis hirsute</i>	animal	40	GrI (0.65)	p_j (0.76)	0.999	0.14***	0.14	0.03	0.21***
<i>Centaurea jacea</i>	animal	76	GrI (0.90)	p_j (0.53)	0.946	0.11**	0.07	0.00	0.12**
<i>Koeleria pyramidata</i>	animal	66	GrI (0.71)	p_j (0.57)	0.815	0.09**	0.05	0.03	0.13*
<i>Linum catharticum</i>	animal	48	GrC (0.81)	p_j (0.58)	1.122	0.18***	0.13	0.04	0.26***
<i>Medicago lupulina</i>	animal	62	GrC (0.95)	p_j (0.58)	1.041	0.15***	0.09	0.03	0.19***
<i>Plantago media</i>	animal	77	GrC (0.82)	n_j (0.53)	1.497	0.19**	0.06	0.08	0.22*
<i>Polygala comosa</i>	animal	48	GrI (0.73)	p_j (0.73)	1.233	0.19***	0.14	0.05	0.28***
<i>Prunella grandiflora</i>	animal	73	GrI (0.97)	p_j (0.6)	1.286	0.20***	0.09	0.10	0.31**
<i>Ranunculus bulbosus</i>	animal	71	GrI (0.83)	p_j (0.69)	1.275	0.20***	0.13	0.02	0.22**
<i>Salvia pratensis</i>	animal	75	GrC (0.43)	n_j (0.37)	0.913	0.10*	0.02	0.08	0.18 n.s.
<i>Scabiosa columbaria</i>	animal	67	GrC (0.96)	n_j (0.84)	1.131	0.21***	0.17	0.01	0.22***
<i>Hieracium pilosella</i>	wind	57	GrC (0.96)	p_j (0.73)	1.391	0.24***	0.21	0.04	0.28***
<i>Leontodon hispidus</i>	wind	54	GrI (0.90)	n_j (0.62)	1.392	0.22***	0.20	0.05	0.31***
<i>Anthyllis vulneraria</i>	wind	28	GrC (0.81)	p_j (0.69)	0.804	0.11**	0.11	0.01	0.14**
<i>Campanula rotundifolia</i>	wind	77	Eu (0.73)	p_j (0.87)	-1.360	0.17**	0.12	0.12	0.24**
<i>Carlina acaulis</i>	wind	62	GrI (0.65)	p_j (0.68)	0.883	0.12**	0.06	0.07	0.21*
<i>Cirsium acaule</i>	wind	52	GrI (0.95)	n_j (0.78)	1.296	0.20**	0.15	0.05	0.27***
<i>Pulsatilla vulgaris</i>	wind	44	GrC (0.5)	n_j (0.53)	0.639	0.07**	0.07	0.05	0.13**
<i>Ajuga genevensis</i>	none	52	GrC (0.6)	p_j (0.43)	0.541	0.05*	0.0	0.13	0.19 n.s.
<i>Asperula cynanchica</i>	none	49	GrI (0.97)	p_j (0.96)	1.184	0.18***	0.19	0.04	0.26***
<i>Dianthus carthusianorum</i>	none	74	GrI (0.84)	n_j (0.59)	1.167	0.19***	0.09	0.05	0.22**
<i>Euphorbia cyparissias</i>	none	77	GrI (0.71)	n_j (0.62)	1.091	0.13**	0.06	0.01	0.1*
<i>Euphorbia verrucosa</i>	none	26	Eu (0.83)	p_j (0.54)	0.551	0.11**	0.13	0.06	0.18**
<i>Hippocrepis comosa</i>	none	66	Matrix (0.67)	p_j (0.54)	-0.748	0.09**	0.14	0.13	0.24**
<i>Ononis repens</i>	none	59	Matrix (0.49)	p_j (0.47)	0.851	0.11***	0.09	0.09	0.22**
<i>Ononis spinosa</i>	none	17	GrI (0.63)	n_j (0.87)	1.254	0.28***	0.30	0.00	0.32***
<i>Onobrychis vicifolia</i>	none	39	GrI (0.93)	p_j (0.79)	0.929	0.13***	0.13	0.01	0.17**
<i>Phleum phleoides</i>	none	34	GrI (0.23)	p_j (0.52)	0.285	0.03 n.s.	0.01	0.14	0.16 n.s.
<i>Stachys recta</i>	none	19	GrI (0.89)	p_j (0.45)	0.940	0.16***	0.15	0.01	0.19**
<i>Trifolium montanum</i>	none	41	GrI (0.57)	p_j (0.56)	1.314	0.24***	0.22	0.03	0.32***

^aNumber of occupied patches for each species from the 2009 survey.

^bTop-ranked distance model (d_{ij}) selected for each species (GrI, consistently or intermittently grazed; GrC, consistently grazed; Eu, geographic distance; Matrix, restricted dispersal through forest and dense vegetation).

^cSummary of best performing S_i index. In parentheses relative importance values of the best models for the distance (d_{ij}) and source patch parameters (p_j , species occupancy; n_j , population size; A_j , patch area).

^dVariation partitioning of unique contributions of the best S_i index and microsite diversity (Elem) to the final model. Statistical significance *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.01$; n.s. $P \geq 0.1$.

Patch Occupancy and Genetic Diversity in *D. carthusianorum*

Multimodel inference showed that GrI best explained both patch occupancy and rarefied allelic richness of *D. carthusianorum* (w_m of 0.84 and 0.74; Figs. 2a and 2c, respectively). For the patch-occupancy data, model selection was not conclusive for any of the 3 source patch parameters (Fig. 2b), whereas for the genetic data, n_j ($w_m = 0.38$) and A_j ($w_m = 0.02$) had lower support than the simplest model that included only p_j ($w_m = 0.6$; Fig. 2d).

Linear regression of A_r on the best-ranked S_i index (GrI and p_j) was significant with an $R^2_{adj} = 0.19$ ($F = 14.22$, 1 and 55 df, $P = 0.0004$). We further categorized popula-

tion size as small ($n < 40$ individuals) or large populations ($n > 40$ individuals). The 2-way ANOVA showed that A_r differed significantly by population size (df = 1, $F = 7.38$, $P = 0.008$), whereas the difference among patch types was only marginally significant (df = 3, $F = 2.19$, $P = 0.08$). There was a significant interaction between predictors (df = 2, $F = 3.38$, $P = 0.04$). Pairwise comparisons indicated populations in core areas had significantly higher A_r (mean = 4.9) compared with small populations in intermittently grazed patches (mean = 4.3, $P = 0.00001$) and small populations in ungrazed patches (mean = 4.4, $P = 0.01$). Also, intermittently grazed patches differed significantly by population size, with lower A_r for small populations ($P = 0.01$).

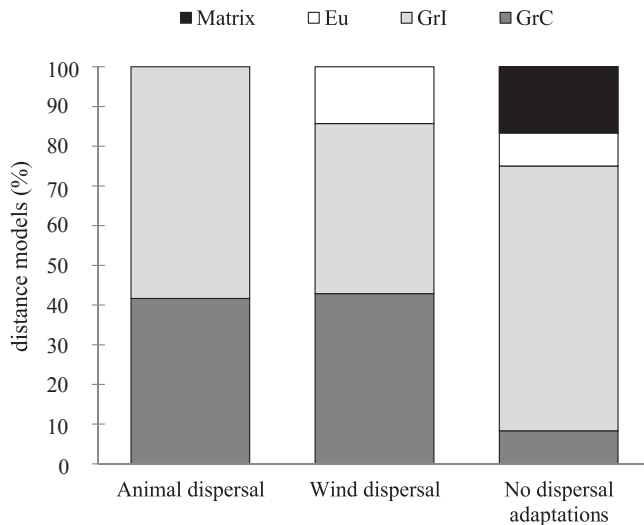


Figure 1. Percentage of each distance model selected within 3 species groups by dispersal mode: animals ($n = 12$), wind ($n = 7$), and species with no specific adaptations for dispersal ($n = 12$) (Eu, geographic distance; GrC, consistently grazed; GrI, consistently or intermittently grazed; Matrix, restricted dispersal through forest and dense vegetation).

Discussion

Interaction Between Shepherding Connectivity and Dispersal Traits

For most of the 31 species, including those not dispersed by animals, effect of shepherding connectivity (GrC or GrI) on landscape-scale patch occupancy was significant. Both GrC and GrI included a distance-dependent effect of shepherding connectivity in terms of the number of patches that sheep traverse along the route. The support of a distance-dependent effect suggests that for most species, a large proportion of seeds are transported to nearby grasslands. This is consistent with results of a field experiment, where $>50\%$ of seeds fell off of sheep wool within the first hours after grazing (Fischer et al. 1996). The matrix resistance and geographic distance models were the best-ranked models for 4 species dispersed by wind or that had no dispersal adaptations. Connectivity studies in calcareous grasslands show that dispersal of many habitat specialists is spatially restricted over short distances regardless of whether they have specialized dispersal adaptations, which makes colonization unlikely among fragmented grasslands (Poschlod et al. 1998; Maurer et al. 2003; Jacquemyn et al. 2011).

Habitat restoration of calcareous grasslands by rotational sheep grazing is expected to support plant dispersal (Fischer et al. 1996; Wessels et al. 2008). The proportion of top-ranked connectivity models did not differ significantly between species with different dispersal modes (animals, wind, or no adaptation); thus, sheep

may act as an important dispersal mechanism for many species. However, species with no dispersal adaptations presented the largest variation in the number of best models selected, which shows the variability of the interaction between the plant and available dispersal vectors. Also, increased ecological drift in fragmented habitat and the stochastic nature of dispersal may explain the relatively low explanatory power of our models. Zoochorous species with longer seeds tended to show a stronger response to shepherding connectivity than other species, though only zoochory had a significant effect. This result, which suggests higher dispersal rates for zoochorous, is consistent with previous studies that found higher probability of seed attachment to wool by specialized seed appendages and by longer seeds that are more likely to be caught in wool (Fischer et al. 1996; Couvreur et al. 2004).

Grazing of sheep may affect ecological conditions of individual patches through gap creation via trampling and biomass removal and thus may indirectly influence plant establishment (Faust et al. 2011). When we restricted the analyses to consistently grazed patches to keep grazing intensity constant, shepherding dispersal models remained the best ranked, which suggests that grazing effects per se are unlikely to explain the observed patterns. Similarly, Wagner et al. (2013) found a significant effect of shepherding connectivity on the relative change of species richness in restored patches from 1989 to 2009 after accounting for the effect of grazing treatments.

Properties of Source and Focal Patches in Species Occupancy

Similar to the findings at the community level (Rico et al. 2012), patch area as a proxy of seed production did not improve model fit relative to the model that included patch occupancy alone. Also, we found that for most species, population size was not a good source patch predictor either. These results indicate that regardless of patch area or population size, presence of a species in source patches may often be sufficient to sustain species presence in local patches (Hanski et al. 2004). Landscape-scale occupancy patterns for most specialist species are thus largely related to the distribution of patches, which act as potential sources, rather than local abundances. This result suggests that restoring connectivity among patches is critical to ensure landscape patch-occupancy in most species.

The diversity of microsites in focal patches also contributed to patch occupancy for most species. Similarly, Wagner et al. (2013) found that microsite diversity affects the increase of species richness more than vegetation type, which may indicate edaphic site conditions and competition with nonspecialist species. Because this was an observational study, our conclusions are limited given the highly stochastic nature of both dispersal and establishment processes, and we cannot rule out confounding

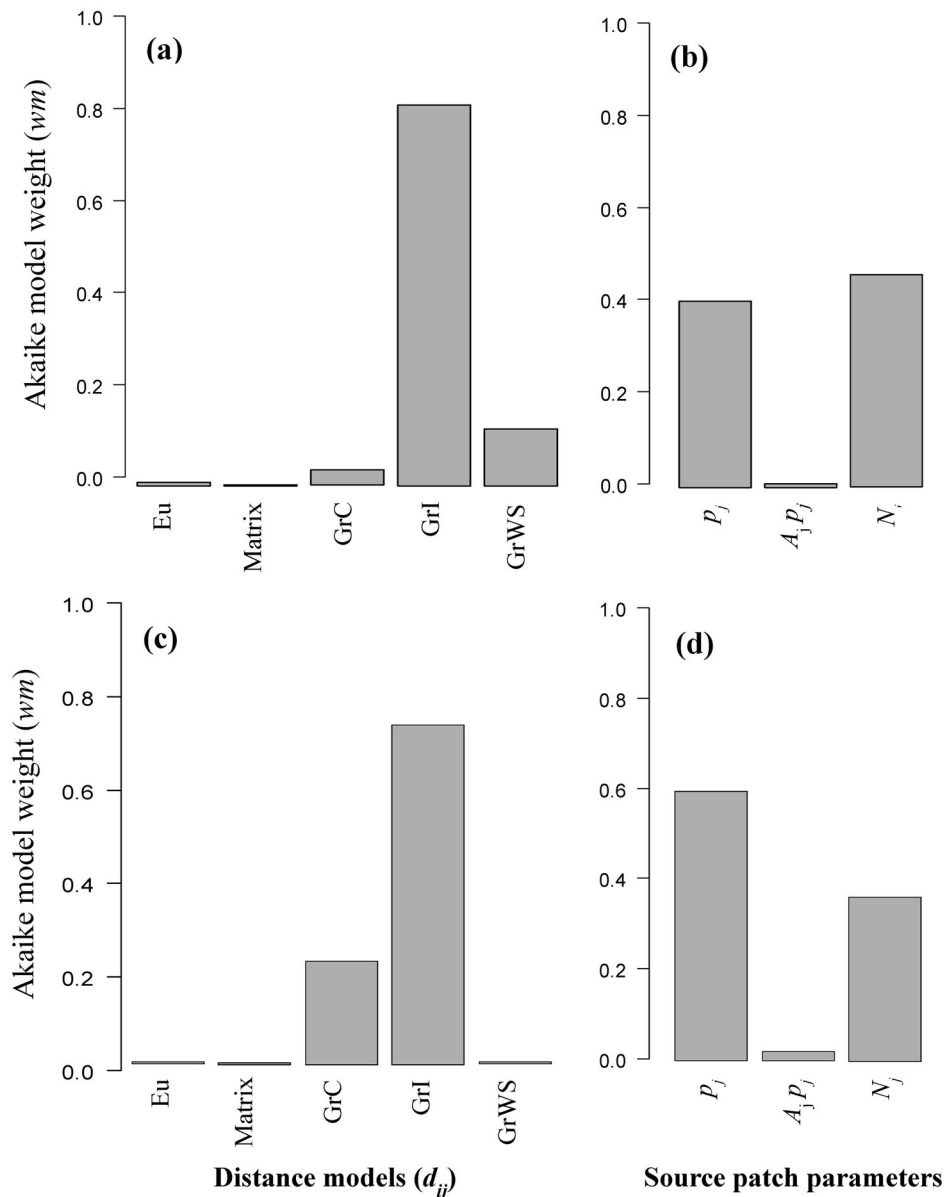


Figure 2. Relative importance of each parameter in the S_i connectivity index for *Dianthus carthusianorum* based on the sum of Akaike model weights (w_m) over all S_i candidate models (m) containing the same parameter. Each bar shows one version of the distance models (d_{ij}) or source patch parameters (p_j , A_j , and n_j) of S_i models for presence or absence (a and b, $n = 96$ patches) and rarefied mean allelic richness data (c and d, $n = 58$ patches) (Eu, geographic distance; GrC, constantly grazed; GrI, consistently or intermittently grazed; GrWS, grazed within the same grazing system; Matrix, restricted dispersal through forest and dense vegetation).

factors. For instance, other dispersal agents, such as wild and domestic animals, machinery, motor vehicles, and humans may transport seeds among grassland patches (Higgins et al. 2003), and additional factors such as establishment, growth, and mortality may also substantially affect patch occupancy (Clobert et al. 2004).

Demographic and Genetic Connectivity in *D. carthusianorum*

Reduced connectivity not only affects plant demography and probability of colonization by reducing seed dispersal rates, but it also has genetic consequences in plant populations (Aguilar et al. 2008). Ecological data alone, such as patch occupancy, cannot reveal whether restoration of connectivity is sufficient to maintain genetically diverse populations. We evaluated the effect of rotational shep-

herding on dispersal and gene flow in a nonzoochorous species by comparing demographic and genetic connectivity models; an in-depth analysis of population genetic structure of *D. carthusianorum* was beyond the scope of this paper. Although genetic connectivity is often assessed from population pairwise genetic distances, the S_i function is useful in modeling genetic connectivity (Keyghobadi et al. 2005; James et al. 2011). We adopted the S_i function to fit connectivity models by using genetic diversity data (Keyghobadi et al. 2005) to make comparable analyses with the patch occupancy data. Analyses with both data types indicated the potential effect of dispersal by shepherding on patch occupancy and genetic connectivity. These results suggest that although *D. carthusianorum* is nonzoochorous, it responds positively to shepherding connectivity.

Genetic diversity is likely determined primarily by population factors such as mating system and population size (Leimu et al. 2006). However, reduced connectivity also decreases genetic diversity irrespective of a demographic bottleneck (Broquet et al. 2012). The interaction between population size and patch type was significant; small populations of ungrazed or intermittently grazed patches had lower genetic diversity than other patch types. In contrast, core areas (i.e., patches that were not abandoned and that typically harbored large plant populations) had higher levels of allelic richness. Our result may be related to time since restoration in 1989. This period may not have been long enough to counteract reduced connectivity among patches embedded in agriculturally intensive landscapes that may restrict movement of specialized pollinators among patches (Bruckmann et al. 2010). Small populations with reduced genetic diversity are thus more likely to experience reduced fitness and population viability, which may affect their persistence in the long term (Leimu et al. 2006; Aguilar et al. 2008). Although our results indicate that population size of source patches might not be an important predictor for species patch occupancy, estimates of genetic diversity of *D. carthusianorum* suggest that managing local population size is still important to maintain genetic diversity. However, our results also suggest that a potential effect of directed seed dispersal by sheep on genetic connectivity remains limited because nuclear markers incorporate gene flow events from both seeds and pollen.

To effectively maintain species and genetic diversity in fragmented landscapes, we need to quantify and understand what determines connectivity. Our analyses in calcareous grasslands suggest a potential effect of rotational shepherding to support dispersal of specialist plants and genetic connectivity in *D. carthusianorum*. Landscape patch occupancy for most species did not depend on the size of source populations and most species responded positively to shepherding connectivity, irrespective of zoochory. In a different context, managers of invasive rangeland species may need to consider domestic animals as important dispersal mechanisms even if the species lacks dispersal adaptation or populations are small.

Our results further suggest that managing connectivity of calcareous grasslands is equally important as managing local patch conditions, such as the presence of microsites increasing probability of species establishment. Our study supports the key role of rotational shepherding to provide dispersal for a wide range of species; thus, shepherding constitutes an effective practice to protect species diversity in fragmented calcareous grasslands.

Acknowledgments

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC

Discovery Grant to H.W.), the National Council on Science and Technology of Mexico (CONACYT to Y.R.). We thank K. Dadrach, D. Baumgartner, B. Raab, S. Haacke, J. Sachteleben, and the shepherds E. Beil, E. Neulinger, and A. Grimm for valuable information. We thank H. Lehnert for field support, R. Graf for lab work support, and 4 anonymous reviewers for their valuable comments to improve this manuscript.

Supporting Information

Optimized α values of 5 d_{ij} distance models for 31 habitat specialist plants of calcareous grasslands (Appendix S1), estimates of genetic diversity for 11 polymorphic loci in 59 grassland patches in *Dianthus carthusianorum* (Appendix S2), and results of sensitivity analyses restricting focal sites to continuously grazed patches for the best performing parameters of the S_i connectivity index (Appendix S3) are available online. The authors are solely responsible for the content and functionality of these materials. Queries, other than the absence of material, should be directed to the corresponding author.

Table S1. Optimized α values of 5 d_{ij} distance models for 31 habitat specialist plants of calcareous grasslands. Bold numbers indicate the α value for the best performing connectivity model.

Table S2. Estimates of genetic diversity for eleven polymorphic loci in 59 grassland patches in *Dianthus carthusianorum*. Patches are defined by population size and patch type as core areas, grazed restored patches (consistently grazed, intermittently grazed), and ungrazed patches. N , sampled size; H_o , observed heterozygosity; H_e , expected heterozygosity; A_r , rarefied mean allelic richness.

Table S3. Sensitivity analysis restricting focal sites to continuously grazed patches for the best performing parameters of the S_i patch connectivity index and relative contribution of patch connectivity (S_i) and microsite diversity (Elem) to the total variance explained of final models of patch occupancy for 31 habitat specialist plants of calcareous grasslands.

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