An orchid colonization credit in restored calcareous grasslands

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Abstract

Habitat restoration comprises the recreation of suitable environmental conditions with the intention of recolonization by certain target species. In previously abandoned calcareous grasslands, however, many characteristic plant species have been reported missing, even decades after the reinstatement of the traditional mowing or grazing management. Such grasslands are said to exhibit a colonization credit. This may be particularly true for orchid species, which often rely on highly specialized pollination strategies and mycorrhizal associations for completion of their life-cycle. In this study, we investigated whether restored calcareous grasslands exhibited an orchid colonization credit, whether this credit was associated with the degree of grassland fragmentation, and with particular species' life history traits. Applying the Beals index as a quantitative method to identify suitable habitats, several orchid species were indeed found missing from grasslands deemed suitable. There was no relation, however, between the extent of the colonization credit and the spatial isolation or size of the grasslands. From all life history traits examined, only a high degree of pollinator specialization could be related to delayed colonization. This may suggest that restoring the pollinator community is an important bottleneck in calcareous grassland restoration.

Keywords: Beals index, habitat suitability, colonization delay, habitat fragmentation, life history traits, specialized pollination, grassland management

Introduction

Temperate grasslands are one of the world's most species rich plant-communities at a small scale (<10 m²). Especially calcareous grasslands are known to be highly diverse, with up to 60 plant species·m⁻² (Kull & Zobel, 1991; Eriksson & Eriksson, 1997). Next to the high plant diversity there is an even greater diversity of arthropod species, which makes these nutrient-poor grasslands regional biodiversity hotspots (WallisDeVries, Poschlod & Willems, 2002; Cremene et al., 2005). Historically, calcareous grasslands were limited to extreme environmental conditions on steep calcareous mountain slopes. Early human activities such as deforestation and increased sheep flock migrations (transhumance) increased their area and maintained these grasslands on calcareous hills all over Western Europe (Poschlod & WallisDeVries, 2002). By the end of the 19th century, free sheep grazing declined steeply due to urbanization, the collapse of the local wool market and intensification of agriculture through fertilizers (Poschlod & WallisDeVries, 2002). Through natural succession, these unattended grasslands slowly started to transform. First species to disappear were annual and shade-intolerant species due to dominance of some grasses (mostly Brachypodium pinnatum or Bromus erectus) (Bobbink & Willems, 1987). As the herbaceous layer grew higher and denser, seed germination and seedling establishment of typical grassland species declined (Delescaille et al., 2005; Jacquemyn et al., 2011), and grasslands species were replaced by shrub and tree species. In many parts of north-western Europe, efforts to restore the overgrown grasslands only started in the 1970s (Delescaille et al., 2005).

Restoration success largely depends on two factors, (i) the recreation and maintenance of suitable environmental conditions, and (ii) the recolonization of target species (Perrow & Davy, 2002, Piqueray & Mahy, 2010). Restored grasslands that are deemed suitable with respect to habitat quality, and that have not yet been fully colonized are said to exhibit a

‘colonization credit’ (Cristofoli *et al.*, 2010). We define colonization credit as the number of species yet to colonize a grassland, following the restoration of habitat quality. In quantifying the colonization credit, it is crucial to be able to determine the habitat suitability for target species. Including direct measures of habitat suitability, such as for example soil characteristics, is not a trivial task as there are many candidate variables, and there is no objective procedure to decide which ones are the most relevant (Münzbergová & Herben, 2004). To meet this problem, measures of habitat suitability that are based on the species composition of all present species in a habitat have been proposed. This approach can be based on a hypervolume approach based on Ellenberg indicator values (Butaye *et al.*, 2002), or on the Beals Index (BI) (Beals, 1984; Munzbergova & Herben, 2004). The application of the BI is based on the general observation that occurrences of species are correlated (Munzbergova & Herben, 2004).

Before a habitat that is deemed qualitatively suitable is successfully colonized, different sequential successes are necessary. First, dispersal of propagules is required, followed by establishment of individuals, and their survival and growth to reproductive individuals (Jackson & Sax, 2010). If any of these steps is delayed or obstructed, it causes a delay in the colonization of a habitat, resulting in colonization credit (Jackson & Sax, 2010). Colonization delays happen frequently and have been demonstrated for non-native species invasions (Kinlan & Hastings, 2005), natural, climate-driven migrations (Menendez *et al.*, 2006; Willis *et al.*, 2009), and for the colonization of newly created habitats (Jacquemyn, Butaye & Hermy, 2003, Cristofoli *et al.*, 2010). Habitat fragmentation can be expected to be an important driver of delayed colonization for two reasons. First, habitat area may hamper successful colonization because small grasslands can only harbour small populations which are more susceptible to local extinction through environmental and demographic stochasticities, natural catastrophes and reduced genetic diversity (Shaffer, 1981). Second,

spatial isolation of habitats is expected to negatively affect both the survival of small populations through impeding a rescue effect (Brown & Kodrick-Brown, 1977) and the (re-) colonization of habitat (Hanski, 1998).

Species richness has been widely used as a biological indicator of restoration success in calcareous grasslands (e.g. Lindborg & Eriksson, 2004), even though it is a rather rough indicator (Kiehl, Thormann & Pfadenhauer, 2006). Other indicators can be useful to evaluate the efficiency of restoration practices (Samu, Csontos & Szinetár, 2008). Orchid species are among the most recognizable members of the plant kingdom. Because they are habitat specialists and are largely dependent on, often specialized, biotic interactions with insect pollinators and mycorrhizal fungi (Waterman & Bidartondo, 2008; Swarts & Dixon, 2009), they can be considered as good indicators of ecosystem health and ecosystem integrity (Swarts & Dixon, 2009). Even among orchid species, however, important trait differences occur, e.g. with respect to plant height, longevity, pollinator and mycorrhizal specialization, and nectar production. Because plant traits are important mediators of a species' responses to habitat deterioration, fragmentation and restoration (Jacquemyn, Brys & Willems, 2005; Adriaens, Honnay & Hermy, 2007; Swarts & Dixon, 2009) it can be expected that there is some variability in the colonization delay among different orchid species.

Here, we investigated whether once deteriorated calcareous grasslands exhibit an orchid colonization credit, more than three decades after the reinstatement of the traditional management. Because, as in many restoration studies, a clear reference habitat is lacking, and restoration sites vary in habitat quality, we applied the BI as a quantitative method to detect suitable grasslands. More specifically this paper aims to:

1. Determine the reliability of the BI for estimating habitat suitability by comparing it with abiotic habitat quality measures;
2. Use the BI to evaluate the presence of an orchid colonization credit by identifying

suitable but unoccupied grasslands;

3. Evaluate whether the colonization credit is associated with small habitat area and low habitat connectivity;

4. Identify orchid life history traits that mediate delayed colonization.

Materials and Methods

STUDY AREA AND SPECIES

The study area roughly coincides with the valley of the river Viroin in the southwestern part of the Calestienne region, Southern Belgium (Figure 1; Adriaens, Honnay & Hermy, 2007). It covers approximately 80 km². The soil has been formed in a southwest to northeast oriented geological belt of Devonian limestone hills. The calcareous grasslands can be found on stony outcrops, on altitudes between 150 and 250 m. The mean annual temperature is 9.8 °C and the area receives a mean annual precipitation of 780 mm (Royal Meteorological Institute Belgium). The studied calcareous grasslands were once part of a larger grazing system with migrating sheep flocks. By the end of the 18th century free grazing practices diminished and are suspected to have stopped completely by 1950. It was not until the 1970s that the first attempts were made to manage and restore the habitat quality of the remnant grassland. Until the 1990s, grasslands were managed dominantly by mowing and after that date grazing became most important (Adriaens, 2008). In an earlier study of these grasslands, Adriaens *et al.* (2006), have been reconstructing the historical area of the individual grassland fragments since 1775, and they were not able to demonstrate a species extinction debt. Here, we adopt a different view on these grasslands, by recognizing that even the grasslands still present today have gone through a period of succession and shrub

encroachment, and that they regained habitat quality only during the last 30 years, through the reinstatement of the mowing and grazing regime.

The presence of herbaceous plant species was surveyed in 64 grasslands. Surveys were conducted in spring and late summer 2004, by walking parallel transects through the entire grassland. Species found were then added to a pre-existing list of 415 1 m² vegetation relevés, recorded in 2003 (Butaye *et al.*, 2005). The number of established relevés was in proportion to the grassland area, ranging between 2 and 30 per grassland.

Only the 120 habitat specialist species that belonged to the typical Festuco-Brometea, Sedo-Scleranthea and Trifolio-Geranieta vegetation types were listed (Butaye *et al.*, 2005). Of these species, only 90 species that occurred in at least five grasslands were retained for further analysis. Of the 20 orchid species occurring in the study area, nine species met these criteria: *Epipactis atrorubens*, *Gymnadenia conopsea*, *Himantoglossum hircinum*, *Listera ovata*, *Ophrys fuciflora*, *Ophrys insectifera*, *Orchis anthropophora*, *Orchis mascula* and *Platanthera chlorantha* (nomenclature according to Lambinon *et al.*, 2004). We classified the species according to nectar production (yes or no), average plant height at inflorescence (short or tall (< or ≥ 30 cm respectively)), growth habit (rhizomatous or tuberous), lifespan (short- or long-lived (≤ or > 3 years respectively)), pollination (specialized or general (≤ or > 10 pollinator species respectively)) and self-compatibility (yes or no) (Table I). Plant traits were retrieved from literature (Van der Cingel, 1995, Kull & Hutchings, 2006, Schatz, 2006).

ENVIRONMENTAL VARIABLES, GRASSLAND SUITABILITY AND CONNECTIVITY

In each relevé, we measured the following environmental variables: percentage cover of bryophytes, rock and bare ground, average vegetation height (cm), soil depth (cm) and slope (%). Total standing biomass was estimated by multiplying vegetation cover with mean vegetation height. All environmental variables were averaged across all the vegetation relevés

established in a grassland.

Using plant coexistence matrices, the BI was calculated according to the following formula (Beals, 1984; Munzbergova & Herben, 2004):

$$p_{ij} = S_i^{-1} \cdot \sum_k N_{jk} \cdot N_k^{-1} \quad [1]$$

where p_{ij} is the probability of finding species j at site i , S_i is the species richness of site i (minus 1 if species j is present), N_{jk} is the number of joint occurrences of species j and k in the predictor sites and N_k is the number of occurrences of species k in the predictor sites. For each orchid species, unoccupied grasslands were defined as suitable if they had a BI higher than the 5th percentile of the cumulative frequency distribution of the BI of occupied grasslands (Munzbergova & Herben, 2004).

For each grassland i the connectivity (C_i) was calculated according to Moilanen and Nieminen (2002) using the formula:

$$C_i = \sum (A_i \cdot A_j) \cdot d_{ij}^{-1} \quad [2]$$

Where A_i is the area of grassland i , A_j is the area grassland j and d_{ij} is the Euclidean distance between grassland i and j

DATA ANALYSES

For each orchid species, the reliability of the BI estimations of habitat suitability was verified by comparing the BI between occupied and unoccupied grasslands using a Mann-Whitney U test. Next, we restricted our analyses to unoccupied grasslands and tested whether the measured environmental variables were related to the suitability of a grassland (1/0) as estimated by the BI. To avoid the problem of intercorrelation, a dimension reduction was performed on the environmental variables, using a Principal Component Analysis (PCA) with varimax rotation. Significance between Principal Components and environmental variables

were determined by calculating Pearson correlation coefficients between principal component scores and environmental variables. We then used a backward stepwise binary logistic regression to relate the Principal Components to grassland suitability. To determine significance, Wald statistics were calculated.

To evaluate the extent of the orchid colonization credit in our study area, observed grassland occupancy was compared with the number of suitable grasslands (as determined by BI), and the average orchid colonization credit across all grasslands was determined. In order to evaluate the effects of grassland fragmentation on the colonization credit, we performed Spearman rank correlations between the orchid colonization credit of a grassland (i.e. number of orchid species missing) and grassland connectivity, and between orchid colonization credit and grassland area. Area and connectivity were not correlated ($p > 0.1$). To identify traits that may mediate the delayed colonization of orchids, orchid traits were linked to their incidence in suitable grasslands, using a Mann-Whitney U test. All analyses were carried out using SPSS 17.0.

Results

Grasslands that were occupied had, on average, a significantly higher BI value than unoccupied grasslands ($p < 0.05$). More importantly, occupied grasslands generally had a high BI, whereas unoccupied grasslands could have both high and low BI value (Figure 2).

The PCA analysis on the environmental variables yielded three Principal Components (PC) with an eigenvalue higher than 1. Together they represented 74% of the total variance (Table I). All environmental variables correlated very significantly ($p < 0.001$) to one of the three PC. Biomass and vegetation height were positively correlated with PC 1. PC 2

correlated positively with slope and percentage of rock, and negative with soil depth. PC 3 was negatively correlated with percentage cover of bare ground, and positively with percentage cover of bryophytes. Unsuitable grasslands were markedly different from unoccupied suitable grasslands with respect to PC 2 (Table II). However, optimal environmental conditions were not identical for all orchid species. Depending on the species considered, either high or low values of PC 2 were deemed appropriate.

Orchid species occupied 20–70% of all suitable grasslands (Figure 3), and on average, half of all suitable grasslands were occupied. The orchid colonization credit of a grassland was independent of both its area and its isolation (both $p > 0.1$; Spearman rank correlations) (Figure 4). Three orchid traits, lifespan, self-compatibility and average height, could not be related to the incidence of orchids in suitable grasslands as there was insufficient variation among the orchid species considered. Nectar production did not significantly influence the percentage of suitable habitats occupied ($Z = 0.26$; $p = 0.90$), neither did habitat preference ($Z = 0.98$; $p = 0.33$) or growth habit ($Z = 0.44$; $p = 0.66$). Orchids with generalized pollination occupied significantly more suitable grasslands than orchids with specialized pollination ($Z = 2.46$; $p = 0.01$). Orchids with specialized pollination were present in approximately 40% of the suitable habitats, while orchids with a high number of potential pollinators could be found in approximately 60% of the suitable habitats.

Discussion

ARE GRASSLAND SUITABILITY ESTIMATIONS RELIABLE?

The calculated BI of the grasslands occupied by orchid species is generally high, confirming their suitability, while the BI of unoccupied grasslands could be both high or low

(Figure 2), suggesting that some of these grasslands are suitable, while others are unsuitable. These results at least indicate that the BI estimations are able to differentiate between habitats, and that they can provide a predictive value of habitat suitability (Münzbergová & Herben, 2004). Although the BI has been recommended as an indicator of habitat suitability (Ewald, 2002; Münzbergová & Herben, 2004; Pärtel, Szava-Kovats & Zobel, 2011) it has never been directly related to environmental conditions. We found a significant difference between suitable vs. unsuitable unoccupied grasslands with respect to the environmental variables (PCs) measured for six orchid species. Three other species showed no relation at all (H. hircinum, O. fuciflora and O. mascula) (Table 3). The low absolute number of grasslands estimated as suitable for H. hircinum and O. fuciflora (Figure 3), however, likely reduced the power of our analysis. Additionally, other unmeasured abiotic variables such as soil nutrient conditions may affect habitat suitability (Jacquemyn, Brys & Hermy, 2003; Pärtel et al., 2007). Our results therefore confirm the high discriminatory power of the BI.

EVIDENCE OF AN ORCHID COLONIZATION CREDIT?

Piqueray et al. (2011a) have recently demonstrated that many typical grassland species were unable to colonize calcareous grasslands, up to 15 years after forests clearance. Our results show that even three decades after the restoration of degraded calcareous grasslands, orchid species are still only present in approximately half of the habitat grasslands that are deemed suitable. If the grasslands used as a reference for habitat suitability would have an extinction debt, our results would overestimate the number of suitable habitats (Piqueray et al., 2011b). However, this is not the case (Adriaens, Honnay & Hermy, 2006), and our data therefore suggest that suitable environmental conditions have been restored.

The restoration is not yet finished, however, due to the absence of characteristic species in a substantial number of suitable grasslands. On average, grasslands were suitable

for 4.2 orchid species, from which 2 have not colonized yet.

IS THE COLONIZATION CREDIT ASSOCIATED WITH HABITAT FRAGMENTATION?

If the observed colonization credit was caused by high extinction rates in particularly small grassland areas, we would have expected to find a higher colonization credit in smaller grasslands, which was not the case. If isolation was responsible for the colonization credit, we would have expected to find a higher colonization credit in more isolated grasslands. Again no such relation was found.

The absence of a relation between orchid occurrence and grassland isolation may be due to the relatively good dispersal capacities of orchid species, making spatial isolation not the primary determinant of orchid occurrence in grasslands that are assessed suitable (Arditti & Ghani, 2000). First, orchids produce excessive amounts of small dust-like seeds. Only a small part of such a seed contains the embryo while the remainder is filled with air. As a result, orchid seeds can remain airborne for long periods and can disperse relatively far (Arditti & Ghani, 2000). Second, the use of migrating sheep flocks between some but not all grasslands could easily eliminate the effects of spatial isolation between those grasslands. Sheep are excellent seed dispersers, both through exo- and endo-zoochory (Adriaens, Honnay & Hermy, 2007; Kuiters & Huiskes, 2010). Once established, a regular exchange of seeds between founder populations is also necessary for the successful establishment of viable populations. Gene flow through seeds can counter the effect of small effective population size and genetic drift through the input of new alleles (Young, Boyle & Brown, 1996; Jacquemyn *et al.*, 2009). To conclude, there is no evidence that habitat fragmentation is the primary cause of the observed orchid colonization credit in calcareous grasslands. The question therefore remains which factors hamper the colonization of grasslands that are assessed suitable based on the BI.

DO LIFE HISTORY TRAITS MEDIATE THE DELAYED COLONIZATION OF ORCHIDS?

Neither nectar production nor growth habit significantly affected the percentage of suitable habitats occupied. Orchids with specialized pollination, however, occupied a significantly lower percentage of suitable grasslands compared to orchids with many potential pollinators, indicating that there is a stronger colonization delay for orchids with specialized pollination. Orchid rarity has often been linked to unique pollinator requirements (Swarts & Dixon, 2009, Phillips *et al.*, 2011a). Seed set of specialised orchids can be limited by the behaviour and ecology of only a single pollinator species, thereby affecting population growth and colonisation ability of a species (Phillips *et al.*, 2011a, b). Numerous factors such as the lack of specific habitat requirement (e.g. nesting sites), the absolute and relative abundance of flowering species (e.g. Rasheed & Harder, 1997), competition with different pollinator species (Bowers, 1986), and even weather conditions are known to influence pollinators in their abundance, ecology and behaviour.

Orchids also have specialised interactions with fungal symbionts and this specialisation can also lead to rarity in orchids (Swarts *et al.*, 2010). Most orchids produce miniscule seeds which cannot germinate or survive the seedling stage unless there are specific mycorrhizae present (Warcupp, 1981, Rasmussen & Rasmussen, 2009). The absence of specific mycorrhizae could therefore also mediate the absence of orchids in suitable grasslands (Otero *et al.*, 2007), and therefore create a colonization credit. However, recent research discovered that many autotrophic terrestrial orchids have a relatively low degree of mycorrhizal specialization (Jacquemyn *et al.* 2010, Phillips *et al.*, 2011b). Additionally, orchids with a high specificity interacted with common mycorrhiza also available to many other orchid species. Therefore, specialization would not necessarily lead to rarity (Jacquemyn *et al.*, 2010) or a colonization delay. Hence it is unlikely that the mycorrhizal

distribution is creating an orchid colonization credit in these grasslands.

FUTURE RESEARCH AND MANAGEMENT

Restoration efforts have successfully recreated suitable habitats and numerous calcareous grasslands species have successfully recolonized the area. However, many characteristic species remain rare and are often absent from suitable grasslands. We found some evidence that pollinator specialisation may affect orchid distribution in fragmented calcareous grasslands. Clearly, further research is required, with a focus on pollinator behaviour and abundance in relation to orchids reproductive output, seed dispersal, germination and seedling survival. In addition, management efforts may already focus on the creation of suitable habitats for important pollinators like bumblebees, solitary bees and digger wasps. In the short term, the number of suitable grasslands occupied could also be increased through deliberate sheep migrations between grasslands occupied by orchids and grasslands that are deemed suitable.

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480 TABLE I. List of plant traits of all species studied. Traits used: 1) Nectar production (y - yes, n - no), 2)
 481 Pollination (g - general, s - specialized), 3) Grow habit (r - rhizomatous, t - tuberous), 4) Average plant height at
 482 inflorescence (s - <30cm, t - $\geq$ 30cm), 5) life-span (l - long-lived (>3 years), s - short-lived (<3 years)), * -
 483 unknown, 6) self-compatibility (y - yes, n - no).

Species	Plant traits					
	1	2	3	4	5	6
<u>Epipactis atrorubens</u>	y	s	r	t	l	n
<u>Gymnadenia conopsea</u>	y	g	t	t	l	y
<u>Himantoglossum hircinum</u>	y	g	t	t	l	y
<u>Listera ovata</u>	y	g	r	t	l	y
<u>Ophrys fuciflora</u>	n	s	t	t	*	y
<u>Ophrys insectifera</u>	n	s	t	t	*	y
<u>Orchis anthropophora</u>	y	s	t	t	l	y
<u>Orchis mascula</u>	n	g	t	t	l	y
<u>Platanthera chlorantha</u>	y	g	t	t	l	y

484

485 TABLE II. Principal component analyses with varimax rotation was used to perform a dimension reduction of the
 486 measured environmental variables. The component matrix shows the relative values of the environmental
 487 conditions, the eigenvalues and percentage of total variance of each principal component.

Variables	PC 1	PC 2	PC 3
Rock (%)	-0.319	0.803	-0.121
Bare ground (%)	-0.124	-0.092	-0.838
Bryophyte (%)	-0.003	-0.341	0.788
Biomass	0.889	-0.315	0.080
Vegetation height (cm)	0.963	-0.031	0.068
Soil dept (cm)	0.273	-0.504	0.262
Slope (°)	0.042	0.850	0.034
Eigenvalue	2.76	1.27	1.15
% of Variance	27	26	20

488

489 TABLE III. Binary logistic regression equations between grassland suitability and the derived PCs for the orchid
 490 species studied.

Species	Regression equation	Model significance
<u>Epipactis atrorubens</u>	-0.410 - 1.666 PC2	<0.001
<u>Gymnadenia conopsea</u>	-0.184 - 2.483 PC2	<0.001
<u>Himantoglossum hircinum</u>		not significant
<u>Listera ovata</u>	-1.632 - 1.322 PC2	<0.05
<u>Ophrys fuciflora</u>		not significant
<u>Ophrys insectifera</u>	-0.712 - 1.352 PC2	<0.001
<u>Orchis anthropophora</u>	-0.738 + 0.603 PC2	<0.05
<u>Orchis mascula</u>		not significant
<u>Platanthera chlorantha</u>	0.862 - 2.239 PC2	<0.001

491

492

493 FIGURE 1. Location of the study area in the Viroin valley. Sampled grasslands are indicated in black (Adriaens,
 494 Honnay & Hermý, 2007).

FIGURE 2. Beals index values plotted for nine orchid species. Values are separated for occupied (grey) and unoccupied (white) grasslands. Based on the species composition of a grassland the BI predicts habitat suitability. A more suitable grassland has a higher BI. The absolute values of the BI dependent on the frequency of the orchids in the database, hence no between species comparisons can be made.

504 FIGURE 3. Absolute number and proportion of suitable grasslands occupied (black) and unoccupied (grey) for the
 505 orchid species studied.

FIGURE 4. a) Scatterplot of orchid colonization credit of each grassland vs. log transformation of grassland area. b) Scatterplot of orchid colonization credit of each grassland vs. grassland connectivity. Colonization credit was defined as the number of species yet to colonize a grassland. No significant correlations were found ($p > 0.1$; Spearman rank correlations).