Experimental establishment of a declining dry-grassland flagship species in relation to seed origin and target environment

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Supporting species persistence may involve (re)connecting suitable habitats. However, for many declining species habitat suitability and drivers of establishment are poorly known. We addressed this experimentally for a declining flagship species of dry grasslands in Germany, Armeria maritima subsp. elongata. In three regions, we sowed seeds from each of eight source populations back to their origin and to eight apparently suitable, but currently unoccupied, habitats close to the source populations. Overall, seeds germinated and seedlings established equally well in occupied and potential sites indicating that suitable habitats are available, but lack seed input. Germination and establishment varied among sowing sites. Moreover, seeds from populations of lower current connectivity established less well in new sites, and establishment was more variable among seeds from smaller than from larger populations, possibly reflecting genetic consequences of habitat fragmentation. Further, establishment across different new environments differed between seeds from different populations. As this was neither related to a home-away contrast nor to geographic or environmental distance between sites it could not clearly be attributed to local adaptation. To promote long-term persistence within this dry-grassland meta-population context we suggest increasing the density of suitable habitats and supporting dispersal connecting multiple sites, e.g. by promoting sheep transhumance, to increase current populations and their connectivity, and to colonise suitable habitats with material from different sources. We suggest that sowing experiments with characteristic species, including multiple source populations and multiple recipient sites, should be used regularly to inform connecting efforts in plant conservation.

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1. Introduction

In recent decades, habitat fragmentation has caused many local extinctions and at the same time reduced the likelihood of natural recolonisations, at least in Central Europe (Fischer and Stöcklin, 1997; Korneck et al., 1998; Lienert, 2004; Honnay and Jacquemyn, 2007). Therefore, the (re-)connection of habitat patches is considered highly important in nature conservation (e.g. Jedice, 1994; Steffan-Dewenter and Tscharntke, 1998; Bugla and Poschlod, 2005; Piessens et al., 2005; Adriaens et al., 2006; Crooks and Sanjayan, 2006; Damschen et al., 2006, 2008; Roy and de Blois, 2008; Brudvig et al., 2009). However, plant species have rarely been targeted while implementing habitat network systems. One reason for this might be that essential baseline knowledge on their regional dynamics, factors affecting habitat suitability and determinants of colonisation in particular, are still poorly understood (Bullock et al., 2002; Ouborg and Eriksson, 2004).

Suitable sites for a focal plant species are often predicted based on the presence of commonly co-occurring species (Münzbergová and Herben, 2004; Römermann et al., 2007). However, for any single species the predictability of occurrence can be low due to dispersal limitation (Ozinga et al., 2005). Furthermore, site characteristics such as levels of light, nutrients, or soil conditions and the availability of micro-sites might also be important (Grubb, 1977; Fischer and Matthes, 1998; Kardol et al., 2008), and the importance of these individual factors may change during the life cycle (Turnbull et al., 2000; Ehrlén et al., 2006). Thus, suitable sites are difficult to be defined a priori (Freckleton and Watkinson, 2002), and suitability needs to be verified by direct experimental evaluation (Fischer and Matthes, 1998; Ouborg and Eriksson, 2004; Münzbergová and Herben, 2005; Ehrlén et al., 2006).
For plants without specific long-distance seed dispersal vectors, seed dispersal is often undirected and covers only short distances (Nathan and Muller-Landau, 2000), which can result in seeds not reaching suitable habitats even when they are in close proximity to extant populations (Ozinga et al., 2009). Once seeds have arrived at a potentially suitable site, their germination and establishment are affected by the interacting effects of environmental conditions, such as those introduced above, as well as by seed quality. In fragmented landscapes, plants in smaller and more isolated populations generally have reduced fitness compared to larger and more connected populations (Lienert, 2004; Leimu et al., 2006). In this context, it appears important to consider whether the size and isolation of populations of seed origin affects seed quality and therefore future population establishment. Furthermore, if populations are adapted to their local environments, seeds from one population might be successful at sites ecologically similar to the original one, but fail in ecologically different sites (Galloway and Fenster, 2000; Joshi et al., 2001; Bischoff et al., 2006; Becker et al., 2006; Raabová et al., 2007; Bowman et al., 2008).

The performance of seeds from different source populations in various unoccupied suitable sites is of interest in the context of regional plant meta-population dynamics, especially if conservation measures such as dispersal corridors aim at enhancing local colonization. However, seed introduction experiments combining multiple seed sources with multiple sowing sites have not been conducted so far. Turnbull et al. (2000) reviewed sowing experiments that tested seed limitation as a cause for limited regional distribution for some 100 plant species. In most of the studies seeds had been introduced into only one or a few unoccupied suitable sites, and none of the studies integrated a meta-population perspective. Other studies included numerous sowing sites when testing for dispersal limitation at different spatial scales, but did not distinguish between different seed source populations (Ericksen and Kiviniemi, 1999; Ehröin and Ericksen, 2000; Münzbergová, 2004; Kiviniemi, 2008). These studies showed that spatial isolation of populations due to habitat fragmentation limits the ability to reach unoccupied suitable sites and thus to sustain regional meta-population dynamics. However, they did not consider effects of size or connectivity of source populations.

We studied whether habitat fragmentation affects regional plant species dynamics by both limiting seed dispersal to unoccupied sites and by influencing the population-specific establishment probability in such sites. As model species we chose the long-lived plant Armeria maritima subsp. elongata in three regions in the state of Brandenburg in northeast Germany, where the species is considered a dispersal-limited flagship of the declining habitat type of sandy dry-grasslands (Ristow et al., 2006). We asked firstly, if the declining species is limited by the availability of suitable sites or by dispersal. We tested the occurrence of potentially suitable habitats by comparing environmental habitat characteristics and germination and establishment of sown seeds in current populations with the ones in apparently suitable, but currently unoccupied sites close-by. We secondly studied the influence of seed origin and its interaction with the target environment on germination and establishment in such unoccupied sites by studying the establishment of sown seeds from multiple source populations of different size and connectivity to multiple, apparently suitable habitats. Thirdly, we tested whether seeds perform better in an unoccupied site if they originate from geographically close or environmentally similar source sites.

We want to give an example of how to provide information relevant for conservation practitioners aiming to conform to ecological and legal obligations of establishing functional habitat networks for conservation (IUCN, 1980; The Council of the European Communities’ Natura 2000 directive, 1992; German federal conservation law of 2002 (Bundesnaturschutzgesetz)).

2. Materials and methods

2.1. Study system

Central European sandy dry-grasslands are nutrient poor habitats of low land-use intensity and species-rich flora and fauna. However, due to nutrient deposition, direct habitat destruction, abandonment, afforestation and fragmentation these habitats are decreasing all over Europe (Riekken et al., 1994; Poschlod and Schumacher, 1998; Poschlod and WallisDeVries, 2002; WallisDeVries et al., 2002). In the German Federal State of Brandenburg, such grasslands used to be common (Krausch, 1968), but are declining steadily and therefore are under federal and European-wide protection (The Council of the European Communities, 1992; Beutler and Beutler, 2002).

Our study species Armeria maritima subsp. elongata (Hoffm.) Bonnier (referred to as A. elongata hereafter), is a long-lived (up to 30 years; Lefèvre and Chandler-Mortimer, 1984), polycarpic, herbaceous rosette plant primarily distributed in the sandy lowland parts of NE Germany and Poland (Meusel et al., 1978). The species also occurs in mesic grasslands and meadows, and in abandoned fields, but most characteristically in semi-dry to xeric sandy grasslands. Plants flower between May and November and produce single flower heads each with 10–200 individual flowers on leafless stems of 0.15–0.50 m height (Jäger and Werner, 2005). Due to a dimorphic incompatibility system, this insect-pollinated species is an obligate outbreeder (Woodell and Dale, 1993). The single-seeded fruits are typically wind-dispersed for a few meters (Philipp et al., 1992: max. 3.5 m for A. maritima subsp. maritima). Longer-distance dispersal is possible by epizoochory, e.g. by sheep (Bugla and Poschlod, 2005; Wessels et al., 2008). Seeds germinate mainly in autumn, but also throughout the winter until spring. Seeds persist in the soil for less than one year (Thompson et al., 1997 (for A. maritima)).

A. elongata, as a Central-European endemic, is declining strongly in Western Germany, and is protected under the German Species Conservation Act. The species is still more common in the Eastern German state of Brandenburg but due to declining numbers and sizes of populations was recently listed as ‘near to threatened’ (i.e., is of advance-warning status) in the state’s Red List of Vascular Plants (Ristow et al., 2006). Moreover, A. elongata was proposed as focal species (sensu Heywood and Iriondo, 2003) for the establishment of regional habitat networks in Brandenburg (Andreas Hermann, Landesumweltamt Potsdam (Federal Environmental State Agency), personal communication), and thus exemplifies anticipatory conservation attention to a formerly common but now declining species.

2.2. Study design

We selected three study regions along a 200 km long west-east gradient in the state of Brandenburg: “Gülpe” (GP; 52°45’N, 12°15’E), “Ludwigsfelde” (LU; 52°18’N, 13°10’E) and “Oder” (OD; 52°30’N, 14°30’E; these coordinates each mark the NW corner of a 5.5 × 5.5 km² area). In autumn 2004, we recorded all 186 existing populations of A. elongata in these regions. The population sizes ranged from 1 to 10,000 flowering individuals with a mean of 589 ± 1582 SD (n = 91). 47.3% of the populations consisted of less than 100 flowering individuals.

Our study design was based on eight pairs of sites in each of the three study regions; one site per pair with an established population and one unoccupied, but potentially suitable site for A. elongata. We considered a site potentially suitable if the species itself did not occur there, but vegetation composition and structure were similar to the ones at sites with current populations. To establish
the design, we randomly selected eight populations within each region as source populations for our sowing experiment (Fig. 1a). In winter 2004–2005 we randomly selected one potentially suitable site of at least 20 m × 20 m size within a radius of 500 m around each source population. Only for one population in the “Oder” region, there was no potentially suitable site within 500 m distance.

2.3. Habitat characteristics

During a snow-free period between December 2004 and January 2005, we recorded in all 23 potentially suitable and 24 currently occupied sites the vegetation structure as mean vegetation height (cm), and ground cover (%) of herb layer (combining living material and standing litter), moss layer and bare-ground in the four blocks per site which also served as sowing blocks (as described below). Winter vegetation height indicated the mean height of standing plant material, including both living and dead parts, and therefore differentiates between more and less productive sites. At the same time, we sowed seeds of the eight source populations per region into each of these blocks. Hence, these winter vegetation data correspond to the conditions under which the germination in early spring took place (for further details of the sowing experiment see Sections 2.6 and 2.7, and Fig. 1b). In May 2005 (referred to as early summer variables), we estimated the same parameters again and also the ground cover (%) of the litter layer this time in one randomly placed 2 m × 5 m block per site. In these blocks, we also recorded the abundances of all vascular plant species following the Londo (1984) decimal scale. We assumed that these early summer variables describe the conditions under which the seedlings established during the season. For each vegetation record, we calculated the mean ecological indicator values after Ellenberg et al. (1991; Jäger and Werner, 2005) for light intensity (L), temperature (T), soil moisture (F), soil alkalinity (R) and nitrogen availability (N) weighted by species abundance while excluding A. elongata and species represented by just one single individual. Mean Ellenberg indicator values are well established indicators of environmental conditions in Central European habitats (Diekmann, 2003; Ozinga et al., 2005).

We summarised our information on different management types in the single variable ‘biomass removal’ as a coarse estimate of land-use intensity at each site. To this end, we estimated the average amount of biomass removed in relative terms, with a maximum of 100 arbitrary units for sites mown more than twice a year; 90 units for sites mown twice, or grazed by sheep or horses; 80 units for sites grazed at low intensity by cattle or mown once a year; 20 units for sites browsed by deer; 10 units for sporadically mown sites, and 0 for unused sites. For sites with multiple management types, each type was weighted with its temporal proportion of the overall management. Within regions, we determined straight centre-to-centre distances of each source population to each of the chosen potentially suitable sites, and to the five nearest current populations of A. elongata.

2.4. Size and connectivity of the source populations

In autumn 2006, we determined the population sizes of all populations as number of flowering individuals. The largest of the source populations had 10,000 flowering individuals, the smallest 70 (mean 886 ± 2026 SD, median 255). Smaller populations could not be considered since individuals had to be excavated for other experiments. Considering the five nearest neighbouring populations, we calculated the connectivity Si of each source population as $S_i = \sum \exp(-d_{ij})N_j$, (after Hanski, 1994) where $d_{ij}$ is the centre-to-centre distance (km) between the source population and the neighbour population of size $N_j$.

2.5. Seed material

In September and October 2004, we randomly collected all single-seeded fruits (henceforth called seeds) of 20–50 mature flower
heads distributed over the whole area of each source population. For each source population we mixed this bulk sample and we partitioned it into 36 batches of 20 randomly selected, apparently viable seeds. We stored seed batches at 4 °C until sowing.

2.6. Sowing experiment

Our sowing experiment served two purposes. To compare germination and establishment between occupied and potentially suitable habitats we sowed seeds of each population back to its source population and to the paired potentially suitable site close-by. To compare germination and establishment of seeds from different source populations in different potentially suitable habitats, we sowed seeds of all source populations of a region to all unoccupied partner sites within the same region (Fig. 1a and b).

Within the sites occupied by the source populations, we marked four 0.37 m × 0.37 m blocks, separated by 1 m from each other, at the margin or between patches of the existing population. These sowing blocks were placed into spots of habitat quality visually comparable to the ones in the core of the local population, thus limiting the risk of testing unsuitable habitat patches within the occupied sites (Münzbergová, 2004; Kiviniemi, 2008). Within each of the four blocks we placed one 10 cm × 10 cm plot at two diagonally opposite corners. Into one of these plots one seed batch from the local source population was sown whereas the second plot served as unseeded control for natural germination and establishment (Fig. 1b).

Within each potentially suitable site we marked four sowing blocks of 1.1 m × 1.6 m at 2 m distance from each other (Fig. 1b). In each block, we marked 11 sowing plots of 10 cm × 10 cm at 40 cm distance from each other. We randomly allocated one seed batch per regional source population to one plot per block. Three further plots per block served as unseeded controls (Fig. 1b). In three potential sites in the “Ludwigsfelde” region, we could only establish three blocks due to shortage of seeds. For the same reason, we only sowed three instead of four replicate seed batches in three of the potential sites for source population LU06. In total, we sowed 805 seed batches with 16,100 seeds in December 2004 and January 2005. Seeds were not transferred between regions. The sowing density of 20 seeds per 100 cm² is within the natural range of seed densities close to maternal plants (B. Seifert, personal observation in the study area in autumn 2004 and 2005).

2.7. Germination and establishment

We determined germination percentages in all 47 sites by counting the seedlings in April 2005, when plants had cotyledons only or one or two primary leaves. These look very characteristically and often carried remains of the calyx, allowing a highly reliable identification of the seedlings. In the 96 control plots at the occupied sites, we recorded a total of only 12 ‘non-experimental’ seedlings, probably from seeds blown in from the adjacent current population. In the 267 control plots in potentially suitable sites no ‘non-experimental’ seedlings were found at all. During a second visit one year later (in May–June 2006), when plants had developed rosettes typically with 4–13 leaves, we assessed the percentage of established plants per sown seed. In one of the potentially suitable sites the experiment was destroyed unintentionally by a farmer prior to the 2006 census, leaving 24 occupied and 22 potentially suitable sites for analysis. We used Spearman’s rank correlation to test whether ranks of site suitability were consistent among the potentially suitable sites for germination and establishment.

<table>
<thead>
<tr>
<th>Colonisation state of habitats</th>
<th>Occupied</th>
<th>Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental variable</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Winter 2004–2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetation height (cm)</td>
<td>10.6 ± 1.2</td>
<td>16.3 ± 2.3</td>
</tr>
<tr>
<td>Herb cover (%)</td>
<td>40.2 ± 4.0</td>
<td>33.1 ± 4.4</td>
</tr>
<tr>
<td>Moss cover (%)</td>
<td>56.0 ± 6.4</td>
<td>58.3 ± 6.0</td>
</tr>
<tr>
<td>Bare-ground cover (%)</td>
<td>12.5 ± 3.2</td>
<td>20.1 ± 3.7</td>
</tr>
<tr>
<td>Early Summer 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetation height (cm)</td>
<td>47.9 ± 1.0</td>
<td>44.5 ± 3.2</td>
</tr>
<tr>
<td>Herb cover (%)</td>
<td>61.3 ± 3.6</td>
<td>55.7 ± 2.7</td>
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<tr>
<td>Moss cover (%)</td>
<td>41.6 ± 6.2</td>
<td>40.3 ± 6.4</td>
</tr>
<tr>
<td>Litter cover (%)</td>
<td>47.0 ± 4.1</td>
<td>35.9 ± 4.8</td>
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<tr>
<td>Bare-ground cover (%)</td>
<td>4.8 ± 1.5</td>
<td>15.2 ± 2.9</td>
</tr>
<tr>
<td>Ellenberg value L</td>
<td>7.6 ± 0.0</td>
<td>7.6 ± 0.0</td>
</tr>
<tr>
<td>Ellenberg value T</td>
<td>6.2 ± 0.1</td>
<td>5.9 ± 0.1</td>
</tr>
<tr>
<td>Ellenberg value F</td>
<td>3.5 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Ellenberg value R</td>
<td>5.5 ± 0.2</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>Ellenberg value N</td>
<td>3.3 ± 0.1</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Removed biomass (rel. units)</td>
<td>49.1 ± 7.1</td>
<td>44.3 ± 8.3</td>
</tr>
</tbody>
</table>

2.8. Data analyses

We compared the environmental conditions in the occupied and potential sites using a linear mixed model with the random factors ‘region’ and ‘habitat pair’ and the fixed factor ‘colonisation state’ (distinguishing the occupied and potentially suitable habitats). To meet the assumptions of normality and homoscedasticity, we transformed the dependent environmental variables when needed.

We compared percentages of germination and establishment in the occupied and potentially suitable sites as odds (i.e., number of successful seeds in relation to number of sown seeds) with a generalised linear mixed model assuming a quasi-binomial error distribution (Crawley, 2007). Significance tests were based on quasi-F values that we calculated as ratio of the mean deviance due to a factor and the mean deviance due to the appropriate error term (Table 2).

To assess meaningful ecological distances between pairs of sites, we first identified relevant environmental variables by testing their relationship with percentages of germination and establishment success of all sown seeds using a multiple regression approach with binomial error distribution. Region, winter vegetation data, averaged weighted Ellenberg values, and removed biomass were included in the full models for both germination and establishment. Summer vegetation structure data were only included in the model for establishment. We reduced the full models by stepwise backward selection based on the AIC index (Crawley, 2007). Since the resulting models exhibited overdispersion, we re-calculated them under the assumption of a quasi-binomial error distribution. Region, winter vegetation data, averaged weighted Ellenberg values, and removed biomass were included in the full models for both germination and establishment. Summer vegetation structure data were only included in the model for establishment. We reduced the full models by stepwise backward selection based on the AIC index (Crawley, 2007). Since the resulting models exhibited overdispersion, we re-calculated them under the assumption of a quasi-binomial error distribution (Crawley, 2007). As coefficient of determination (a R² analogue ranging from 0 to 1) we calculated a log likelihood ratio R² (further named R²(E)) as 1 – [log likelihood (fitted model)/log likelihood (null model)] (Menard, 2000).

The final minimal adequate model for germination explained a large proportion of the variation (R²(E) = 0.75), with significant
effects of winter vegetation height, winter cover by mosses, bare-ground cover in winter, and mean Ellenberg value R for soil alkalinity. To assess the ecological distance between different sites with respect to conditions relevant for seed germination, we applied a principal component analysis (PCA) of standardised values of the variables in the minimal adequate model. We calculated the ecological distances among pairs of sites per region as Euclidean distances in the space spanned by the first three PCA-axes, which had a cumulative proportion of explained variance of 0.94. As none of the environmental variables explained significant variation in the factor they characterize, i.e., size and connectivity of source population against source population and the other covariates against the source population by sowing site interaction.

We performed all statistical analyses with the statistical software R 2.5.1 (R Development Core Team, 2007).

### 3. Results

#### 3.1. Suitability of unoccupied potential habitats

Overall, environmental characteristics of the selected potential sites were very similar to the ones found in the occupied sites (Table 1). Only mean vegetation height in winter was higher in the potential compared to the occupied sites, and the potential sites had a higher percentage of bare-ground (Table 1). These two variables turned out to be related to germination, but not to establishment, as germination was higher in sites with higher winter vegetation and higher bare ground cover (both p < 0.05). However, two other environmental variables that influenced germination (i.e., winter cover by mosses and mean Ellenberg value for R, see Section 2.8) did not differ between occupied and potential sites (Table 1).

Sown seeds germinated and established in each of the occupied and potential sites in all regions. Of the seeds sown to their home population or to the paired potential site close-by, 10.6% germinated within the first 3–4 months after sowing, by spring 2005. By 2006, 5.6% of these sown seeds had developed into juvenile plants. While germination and establishment varied among regions and habitat pairs, they did not differ between occupied and potential sites (Table 2, Fig. 2) demonstrating the suitability of potential sites for *A. elongata*. The significant habitat pair by colonisation state interaction for establishment reflects that seeds established better in the source site than in the partner site for some habitat pairs and vice versa for others (Table 2, Fig. 2).
3.2. Effects of source population and sowing site on germination and establishment

Of the seeds of all origins sown to the unoccupied potential sites 10.0% germinated in the first 3–4 months, and 4.9% had established one year later. Most of the variation in germination and establishment in the potentially suitable sites was due to environmental variation among sowing sites (Table 3). However, sites of high germination did not necessarily turn out as sites of high establishment (Fig. 3; Spearman rank correlation $R_{GP} = 0.33$, $n = 8$; $R_{LU} = 0.54$, $n = 7$; $R_{OD} = 0.54$, $n = 7$; $p > 0.05$ for each region). While source populations varied in their overall germination success, this was independent of the size and connectivity of the source populations. However, seeds from more connected source population tended to established better (Pearson correlation $R = 0.38$, $p < 0.07$, Fig. 4a; Table 3). Moreover, while mean establishment was not affected by source population size (Table 3), the proportion of established seedlings per source population varied the more among potential sites the smaller the source population was (Pearson correlation $R = −0.42$, $p < 0.05$, Fig. 4b).

Neither home-away contrast nor geographical or ecological distance between source and sowing sites significantly affected germination (data not shown). Similarly, neither home-away contrast nor geographical distance between source and sowing sites significantly affected establishment (Table 3). Thus, the significant interaction between source population and potential site for establishment indicates idiosyncratic effects of specific combinations of source population and habitat type, which cannot be explained by local adaptation (Table 3).

4. Discussion

4.1. Availability of suitable unoccupied habitat patches

The environmental similarity of the surveyed occupied and potential sites and the successful establishment of sown seeds in both types of sites indicate that suitable habitats are available in the vicinity of occupied habitats. Our experiments covered germination and survival during the first year which are generally considered critical phases for population establishment. For *Armeria maritima* (Mill.) Willd., survival is limited for seedlings (29%), but increases from year to year to 87% for 4–5-year-old-plants (Lefèbvre and Chandler-Mortimer, 1984). Increasing survival with increasing plant age implies that assessing early survival is likely to provide a measure of survival at a critical stage in population establishment. Moreover, about 20% of our experimental plants had grown to a size sufficient for reproduction (according to our own unpublished observations in numerous populations within the study regions), and three individuals had already developed inflorescences in the first year indicating a short pre-reproductive time. We conclude that suitable habitats are available and the distribution of this species is seed limited (Turnbull et al., 2000; Ehrlén et al., 2006).

Repeated population surveys in 2004 and 2006 showed that colonisation events occurred mainly in areas with rotating sheep pasturing (own unpublished data). This supports that sheep are likely to be a main dispersal vector (Bugla and Poschlod, 2005; Wessels et al., 2008). However, free-ranging or herded livestock grazing has become rare in Northwest-Europe (Ozinga et al., 2009). Likewise, rural structures such as unpaved road verges serving as corridors and open and abandoned sandy dry-grassland sites in villages providing main or stepping-stone habitats have declined due to increasing development during the past 20 years (Ristow et al., 2006). The re-establishment of such landscape links could be very valuable in supporting the connectivity of sandy dry-grassland populations.

4.2. Environmental and potential population-genetic determinants of successful population establishment in a new habitat

Seedlings established in all the tested potential sites. Among those some sites provided better conditions for germination and establishment than others (Table 3). However, good sites for germination were not necessarily also beneficial for establishment.
Thus, suitable habitats may best be environmentally heterogeneous to support successive stages of population establishment in *A. elongata*, as it has been shown also for other plant species (Turnbull et al., 2000; Ehrlén et al., 2006). A main feature of our comprehensive sowing experiment was studying the interplay between seed origin and sowing site. Seeds did not perform better when sown more closely to their home site, neither when we compared seed sources within and between habitat pairs (i.e., testing for a “home-away” contrast) nor when we tested for geographic or environmental distance effects. These findings indicate that populations of *A. elongata* are not adapted locally, at least not at the <8 km scale within our study regions.

The lack of local adaptation may have several reasons. Possibly, within our study regions environmental differences among the study populations in sandy dry-grasslands were not strong enough to induce local adaptation. On the other hand, local adaptation was reported at similar spatial scales for species with similar attributes, i.e., formerly more common fragmented species with outcrossing mating system and low dispersal ability (Raabová et al., 2007; Bowman et al., 2008). Alternatively, though gene flow in *Armeria maritima* was reported to be limited over small spatial scales (<1.3 km: Philipp et al., 1999; but see Philipp et al., 1992; 8–30 km: Baumbach and Hellwig, 2003) it may still have been sufficient to prevent local adaptation (Kamm et al., 2009) of our study.

![Fig. 3. Germination (a) and establishment (b) of seeds from eight regional source populations (columns within a plot) sown to each of the eight (seven for OD) potential sites (rows within a plot) within their region (GP, LU, OD). Within each plot, i.e., within each region, source populations were ordered according to their size, with the smallest population to the left. Dot size increases with the proportion of successfully germinated or established seeds. Note that the dot size scale for germination (from 0.00 [smallest dot] to 0.52 [largest dot]) differs from that for establishment (from 0.00 to 0.21). Black dots indicate the “home” situation where we had sowed seeds within the same habitat pair (Fig. 1) and grey dots indicate seeds sown to other habitat pairs. In region LU, potential site “01” was destroyed after germination.](image)

![Fig. 4. (a) Relationship between the connectivity of the 24 source populations and the mean establishment of seeds originating from these populations each sown to eight potentially suitable sites (Pearson correlation: $R = 0.38$, $p < 0.07$). Shown are the residuals after correction for the effects of ‘region’ and ‘population size’ of the source populations (see Table 3). Connectivity was based on the distance to and the size of the five closest neighbour populations (see Section 2.4). (b) Relationship between the log-transformed size of the 24 source populations and the among-target-site variability of the establishment of seeds from these source population in eight potentially suitable sites (shown as standard deviation SD; Pearson correlation: $R = -0.42$, $p < 0.05$).](image)
populations. Finally, according to a recent meta-analysis of reciprocal transplant experiments (Leimu and Fischer, 2008) the small size of our study populations (median: 255 flowering individuals) could have rendered local adaptation unlikely. In that review, lack of local adaptation of populations smaller than 1000 flowering individuals emerged as general pattern and was attributed to low genetic variation and therefore lacking adaptive potential of small populations (Leimu and Fischer, 2008).

The interaction between site of origin and target site found in our study (Fig. 3) indicates that variation among source populations in their response to new target sites co-determines the success of establishment. As this was not related to local adaptation it may be due to specifics of individual environmental or genetic population histories (Leimu et al., 2008). In any case, it implies that sowing seeds of only one seed source to suitable habitats does not guarantee successful establishment of the target species, if seed origin and target environment do not match.

Seeds from less connected populations tended to establish worse in new sites than seeds of more connected ones. Moreover, seeds from smaller source populations showed higher variability in their response to different new sites than seeds from larger populations. One possible reason underlying these findings could be erosion of genetic diversity of small and isolated populations and subsequently reduced fitness and capability to deal with varying environments (Leimu et al., 2006). We do not have molecular or quantitative-genetic data on the genetic diversity of our very study populations. In an isozyme study involving 17 Danish Armeria maritima populations with a few to 300 km distance Weidema et al. (1996) found reduced gene diversity of small populations, and also higher genetic differentiation among small populations (<100 individuals) compared with medium (101–1000) or large (>1000) ones. Their gene-flow estimate corresponded to other outcrossing animal-pollinated species. However, this gene flow was obviously not sufficient to counterbalance loss of genetic variation from small populations. Although we consider it likely that the small and less connected populations in our study also are of reduced genetic diversity, which might suggest negative fitness effects (Leimu et al., 2006), it can be noted that the observed better ability of more connected and larger populations to cope with new environments was not very pronounced in the current study.

4.3. Conclusions

Our study of a flagship species of fragmented grasslands exemplifies that populations of declining species can be very close to suitable unoccupied habitats, which are not colonised due to seed dispersal limitations. This underlines that availability of, and dispersal between, sites of adequate habitat quality should be recognised as key factor for the regional distribution of dispersed-limited plant species (Primack and Miao, 1992; Ozinga et al., 2005). An important general conclusion emerging from our study therefore is that dispersal networks for such dispersed-limited plant species must include much smaller spatial scales than current network approaches are doing, which are often optimised for large mobile animals (Ozinga et al., 2009). As we neither detected strong effects of seed source nor evidence for local adaptation, adaptation considerations appeared less important, at least at the considered spatial and temporal scales. However, the revealed significant interplay of seed origin and local environment in determining the establishment success in a new site indicates that seed origin still plays a role. Moreover, although not very pronounced in our study, the greater establishment success of better connected populations and the larger variation between seeds from smaller than larger populations support that negative genetic effects of habitat fragmentation must not be neglected when taking conservation measures (Oostermeijer et al., 2003).

We conclude that conservation of the studied dry-grassland flagship species requires either active support of dispersal to suitable sites or a further increase in the density of suitable habitats. Colonisation can be promoted by enhancing the number of arriving seeds, for example by sheep herding or habitat corridors that would connect existing populations with unoccupied and suitable patches. An improved dispersal infrastructure would also enhance genetic exchange among existing populations. According to our results such dispersal networks need to connect multiple populations of seed origin with multiple suitable sites to increase the probability of a good match between arriving genotypes and environment. This approach would at the same time meet the recent criticism of current restoration guidelines for using not only local seed but seed with an appropriate, i.e., in most cases diverse, genetic predisposition (Wilkinson, 2001; Broadhurst et al., 2008).

Our study exemplifies how the combination of environmental information with a sowing experiment involving multiple source populations and multiple sowing sites can clarify habitat suitability and the importance of environmental and population-specific determinants for the ability to establish in new sites. Such regional-specific information, possibly in combination with a metapopulation model, can help to develop the optimal strategy for improving habitat connections in the landscape. We conclude that such experiments should be performed prior to intense, costly and possibly hazardous connecting efforts.

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