http://dx.doi.org/10.1016/j.baae.2005.04.004 (Elsevier)
Basic and Applied Ecology (2006) 7:71-81

Bottlenecks and spatiotemporal variation in the sexual reproduction pathway of perennial meadow plants

Eelke Jongejans[1], Merel B. Soons[2] and Hans de Kroon[3]

1. Nature Conservation and Plant Ecology group, Wageningen University, Bornsesteeg 69, NL-6708 PD Wageningen, the Netherlands. Current address: Department of Biology, The Pennsylvania State University, 208 Mueller Laboratory, University Park, PA 16802, USA.
2. Plant Ecology group, Utrecht University, Sorbonnelaan 16, NL-3584 CA Utrecht, the Netherlands. Current address: Landscape Ecology group, Utrecht University, Sorbonnelaan 16, NL-3584 CA Utrecht, the Netherlands.
3. Department of Ecology, Radboud University Nijmegen, Toernooiveld 1, NL-6525 ED Nijmegen, the Netherlands.

Abstract

Sexual reproduction is important for the growth of populations and the maintenance of genetic diversity. Several steps are involved in the sexual reproduction pathway of plants: the production of flowers, the production of seeds and the establishment of seedlings from seeds. In this paper we quantify the relative importance and spatio-temporal variability of these different steps for four grassland perennials: *Centaurea jacea*, *Cirsium dissectum*, *Hypochaeris radicata* and *Succisa pratensis*. We compared undisturbed meadows with meadows where the top soil layer had been removed as a restoration measure. Data on the number of flower heads per flowering rosette, the numbers of flowers and seeds per flower head, and the seedling establishment probabilities per seed were collected by field observations and experiments in several sites and years. Combination of these data shows that *H. radicata* and *S. pratensis* have higher recruitment rates (1.9 and 3.3 seedlings per year per flowering rosette, respectively) than the more clonal *C. dissectum* and *C. jacea* (0.027 and 0.23, respectively). Seedling establishment is the major bottleneck for successful sexual reproduction in all species. Large losses also occurred due to failing seed set in *C. dissectum*. Comparison of the coefficients of variation per step in space and time revealed that spatio-temporal variability was largest in seedling establishment, followed closely by flower head production and seed set.

Keywords: *Centaurea jacea*; *Cirsium dissectum*; flower production; grassland; *Hypochaeris radicata*; seed predation; seed production; seedling establishment; *Succisa pratensis*

Introduction

The demography of plants comprises the survival and growth of individuals as well as their vegetative and sexual reproduction (Harper 1977). In perennial plants it is mostly survival and vegetative reproduction of existing individuals that contribute to year-to-year population survival (Eriksson 1989; Picó & Riba 2002), but sexual reproduction can markedly increase population growth rate in many cases (Silvertown, Franco, Pisanty & Mendoza 1993; de Kroon, van Groenendael & Ehrlén 2000). In addition, sexual reproduction has merits other than contributing to local plant numbers: it maintains genetic diversity, may form seed banks and enables the population to colonize areas outside the range of vegetative growth (Crawley 1997).

Sexual reproduction, however, is much harder to assess than the other aspects of perennial demography. This is due to the multitude of steps involved and the sometimes very low probabilities of survival from one step to the next. The sexual reproduction pathway generally comprises four steps: (i) the production of flowers and (ii) seeds by flowering plants, and (iii) the establishment of these seeds as seedlings and (iv) reproductive adults (Fig. 1). Many of these aspects have been studied in isolation: research on seed production has focused for instance on effects of population size (Oostermeijer, Luijten, Krenová & den Nijs 1998; Soons & Heil 2002; Brys, Jacquemyn, Endels, Van Rossum, Hermy et al. 2004) geographic distribution (Jump & Woodward 2003; Lienert & Fischer 2004), pollination (Menges 1995; Herrera 2000), seed abortion (Berg 2003), and number-mass trade-offs (Cheplick 1995). Predation by insects on flower heads has been shown to limit seed set (Szentesi & Jermy 2003) and seedling establishment (Louda & Potvin 1995). Research on seedling establishment has mainly focused on the question of whether seeds, microsites or both are limiting seedling numbers (Eriksson & Ehrlén 1992; Jakobsson & Eriksson 2000). Others have compared experimental treatments, concluding for instance that the removal of moss or litter layers and the creation of gaps of bare soil in the vegetation can enhance seedling numbers (Kotorová & Lepš 1999; Isselstein, Tallowin & Smith 2002; Olson & Wallander 2002; Jensen & Gutekunst 2003; Špačková & Lepš 2004).

Assessment of the full sexual reproduction pathway and identification of the bottlenecks for sexual reproduction, however, is only possible when all steps in the sequence are investigated. Only few studies have attempted to compare all subsequent steps of the sexual reproduction pathway in the same system. Meyer and Schmid (1999) highlighted all steps in the clonal *Solidago altissima* and concluded that seedling establishment was very rare. Lamont and Runciman (1993) found that both seed production and seedling establishment were enhanced by fire in two kangaroo paws (Haemodoraceae), while Klinkhamer, de Jong and van der Meijden (1988) concluded that any seed loss will affect their *Cirsium vulgare* populations.

Such studies are more common in forest ecology (Schupp 1990), but the number of studies that analyze all reproduction steps and their variability is particularly low for perennial forbs. In this study we quantify all major steps of the sexual reproduction pathway up to seedling establishment after 12 months to identify the bottlenecks. Also, we compare the temporal and spatial variation in all these steps. As study systems we use four perennial plant species that co-occur in nutrient-poor meadows in the Netherlands (Soons, Messelink, Jongejans & Heil 2003; Hartemink, Jongejans & de Kroon 2004). Specifically we address the following questions: What are the bottlenecks in the sexual reproduction pathway of these perennial grassland herbs? Which steps have the highest temporal and spatial coefficients of variation and are therefore important for the predicted variation in seedling numbers?

Materials and methods

Study systems

We studied four perennials of nutrient-poor meadows: the three Asteraceae *Hypochaeris radicata* L., *Cirsium dissectum* (L.) Hill and *Centaurea jacea* L. s.l. and the Dipsacaceae *Succisa pratensis* Moench. *Hypochaeris radicata* is short-lived (rarely more than two or three years) compared to the other species (potentially decades), which have different strategies of persistence: high rosette survival (*S. pratensis*), formation of new rosettes (*C. jacea*) or rhizomatous, clonal growth (*C. dissectum*) (Hartemink et al. 2004). *Cirsium dissectum*, a rare and endangered (Red List) species, flowers in June, whereas *H. radicata* and *C. jacea* have a more prolonged flowering season. *Succisa pratensis* flowers relatively late, starting in August. All four species produce flowers in flower heads with potentially one seed per flower. The seeds have similar mass (Soons & Heil 2002) and do no form seed banks (Thompson, Bakker & Bekker 1997). The seeds of *H. radicata* and *C. dissectum* have a pappus that may enhance dispersal by wind (Jongejans & Schippers 1999; Mix, Picó & Ouborg 2003; Soons, Heil, Nathan & Katul 2004; Schippers & Jongejans 2005).

The study sites are in the central-eastern part of the Netherlands and are clustered in four regions: Gelderse Vallei (52°01' N, 5°35' E), Veluwe (52°17' N, 5°44' E), Kop van Overijssel (52°37' N, 6°10' E) and Achterhoek (51°59' N, 6°25' E). The range of sites includes nutrient-poor, undisturbed meadows and restoration areas, where the top soil layer had been removed.

Flower head production

Flowering was monitored in three to eight permanent 1 m^2 plots in each of three meadow populations of *C. jacea* (sites B, N and O), *C. dissectum* (sites B, V and O) and *H. radicata* (site L, N and O), and in five meadow populations of *S. pratensis* (sites B, L, V, N and O). In these plots the number of flowering rosettes and number of flower heads per flowering rosette were counted once every year after peak flowering from 1999 to 2003 as part of a demographic study on these species (Jongejans & de Kroon 2005).

Flower production and seed set

Flower heads of the four species were collected in several populations. The criteria for selecting flower heads were that seeds had to be ripe enough to determine their viability, that no seeds had disseminated and that in the case of *C. jacea* and *C. dissectum* all flowers or pappus rings were still present. If one or more of these criteria were not met after collection, the flower head was discarded from the data set. The number of seeds that seemed viable (testing for a hard embryo by squeezing gently with a pair of tweezers) and the number of flowers were counted. Flowers were counted directly in *C. jacea*, but estimated by the number of pappus rings in *C. dissectum* and by summation of the number of viable, empty and predated seeds in *H. radicata* and *S. pratensis*. Furthermore it was evaluated whether any seeds in a flower head had been predated or not. Signs of predation were: crumbled or partly eaten seeds and the presence of insect larvae. The impact of predation in a population was evaluated by comparing the mean number of seeds produced per flower of non-predated flower heads with the mean number of seeds produced per flower of all flower heads.

Seedling establishment

To study establishment probabilities per seed we placed seeds within the vegetation on the moss or soil layer in untreated plots of 5 by 50 cm in November 1999 and November 2000 and counted the increase in rosette numbers compared to changes in rosette numbers in similarly sized control plots (no seeds added). In each plot 100 seeds of a single species were added, which were collected at the same site in the preceding summer. For *C. dissectum* only 50 seeds per plot were used, because of their scarcity. This seed addition experiment was performed at three meadow sites: Bennekomse meent (B), Konijnendijk (N) and Koolmansdijk (O). At site N all four species were used, but *C. jacea* and *H. radicata* were not sown in 1999. At site B all species but *H. radicata* were sown in both years. And at site O only *C. jacea* and *H. radicata* were sown (both years). Per site a full experimental design was made with species, sowing year and treatment (control or seed addition). Within each of the 10 blocks (replicates) the plots were positioned at 20 cm intervals. The blocks were 1 m apart.

In order to investigate a larger range of sites we performed a second seed addition experiment in autumn 2000 at 10 sites: seven undisturbed meadows and three meadows where the top soil was removed by sod-cutting. The set-up was the same as that of the first seed addition experiment, but the number of *C. dissectum* seeds in each plot was increased to 70 and the number of blocks per site was eight instead of ten. In contrast to the previous experiment a mixture of seeds collected from different sites was used per species for all 10 sites, since the species did not occur at all sites. After this experiment we removed all established plants to prevent genetic contamination by the introduced seeds.

Pathway analysis

To assess the overall importance of all steps in the sexual reproduction pathway, we calculated the average number of seedlings produced by a single flowering rosette. To do this, we multiplied the consecutive steps: the mean number of flower heads per flowering rosette, the mean number of flowers per flower head, the number of seeds produced per flower, the fraction of seeds that were not predated and the average fraction of seeds that established as one-year-old seedlings. For each of these steps and for each species separately, we used the mean of the averages of all available site-year combinations. The standard deviation of the resulting number of seedlings per flowering rosette was determined using the following equation for the variance of the product of independent variables (Goodman 1960):

$$\mathrm{var}(\overline{xy}) = \mathrm{var}(\bar{x})\mathrm{var}(\bar{y}) + \bar{x}^2\,\mathrm{var}(\bar{y}) + \bar{y}^2\,\mathrm{var}(\bar{x}) \qquad (1)$$

For these calculations the results of the second seed addition experiment were not used because there was no temporal variation in the data.

To analyze the observed variation in the sexual reproduction pathway, we compare the CVs in space and time for each step and for each species separately:

$$CV_{temporal} = \frac{\frac{1}{f}\sum_{m=1}^{f}(s_{temporal,m})}{\bar{x}_{means}} \qquad (2)$$

$$CV_{spatial} = \frac{\frac{1}{g}\sum_{n=1}^{g}(s_{spatial,n})}{\overline{x}_{means}} \quad (3)$$

where for each species-step combination 'means' is the mean of all site-year means of a species-step combination, f the number of sites with observations in at least two years, g the number of years with observations in at least two sites, $s_{temporal,m}$ the standard deviation between the year-means in site m, and $s_{spatial,n}$ the standard deviation between the site-means in year n.

Results

Flower head production

The number of flower heads per flowering rosette differed significantly between specific site-year combinations in all species (Tables 1 and 2). However, the main effects of site and year were only significant in *Centaurea jacea*, for which 2000 was a year with many flower heads.

Flower production and seed set

On average *Cirsium dissectum* had a significantly larger number of flowers per flower head than the other three species, and *C. jacea* more than *Succisa pratensis*. *Hypochaeris radicata* was intermediate to the last two species and differed only significantly from *C. dissectum*. For each species, site-year interactions were significant (Table 1): the flower heads of *H. radicata* for instance had few flowers in site O only in 2000. In *C. dissectum* there was a main effect of year: in 1999 flower heads contained fewer flowers than in other years. In *C. jacea* and *S. pratensis* the main differences between sites were significant.

The fraction of flowers that set seed in a flower head was positively correlated with flower number in all species, but this correlation existed only as a trend in *C. dissectum* (Table 1). Seed predation significantly reduced the number of seeds produced per flower in all species, but especially in *C. jacea* (Table 1). In this species insect larvae regularly consumed a large part of the developing seeds. Significant site by year interactions and main effects of sites were present in most species. The main effect of year was only significant in *C. dissectum*: 1999 was also a bad year for seed set in this species.

Seedling establishment

Seedling establishment and survival to 12 months after seed addition was highest in *S. pratensis* and *H. radicata*. The first seed addition experiment, which was restricted to undisturbed meadows, revealed significant effects of seed addition only in these two species (Fig. 2A; Table 1). The second experiment, which also included meadows of which the top soil had been removed, showed seed addition effects for all species. In the very open habitats (top soil removed) establishment was higher than in undisturbed meadows for *C. jacea* and *H. radicata* and slightly so in *C. dissectum*, but not in *S. pratensis*.

Full sexual reproduction pathway

When the different steps of the sexual reproduction pathway are compared quantitatively (Fig. 3A,B,C,D), the largest losses take place during seedling establishment. Striking decreases also occur in *C. dissectum* in the seed production step. In *H. radicata* and *S. pratensis* the average number of seedlings that result from these calculations are larger than unity (respectively 1.9 and 3.3), indicating that each flowering rosette would produce one or more one-year-old rosettes via seeds on a yearly basis. In *C. jacea*, however, the calculated seedling number was lower than unity (0.23) and in *C. dissectum* even less: 0.027.

The temporal and spatial coefficients of variation (CVs) showed in which step of the sexual reproduction pathway the highest variation occurred between years and between sites, both compared to the overall mean value of that step in a particular species (Fig. 3E,F,G,H). The highest CVs were those of the spatial differences in seedling establishment. Although based on a small sample of years and sites compared to data on the other steps, variation in establishment probabilities between meadows in the second experiment was as high as that in the first. The CVs of the temporal differences in seedling establishment were also relatively high, as were the CVS of the spatio-temporal differences in the number of flower heads per flowering rosette in *C. jacea* and *S. pratensis* and in the number of seeds produced per flower in *C. dissectum*.

Discussion

Bottlenecks in the sexual reproduction pathway

The most important bottleneck in the sexual reproduction pathway of all four studied perennials was the establishment of seedlings, followed by flower production and species-specific bottlenecks such as seed set in *Cirsium dissectum*, as indicated by the slopes in Fig. 3. The seedlings were not followed from the autumn of the year after seed production until they flowered themselves, but we have probably captured the most important bottlenecks as Jongejans & de Kroon (2005) estimated that one-year old seedlings have high survival rates (90%, 54% and 86% for *Centaurea jacea, Hypochaeris radicata and Succisa pratensis,* respectively). We found the lowest recruitment rate in *C. dissectum* and *C. jacea* which both show extensive clonal growth. We calculated that each flowering rosette has a chance of producing a seedling of only 0.23 (*C. jacea*) or 0.027 (*C. dissectum*) per year. However, clonal species need less recruitment per year to maintain population size and, more importantly, to maintain genetic diversity because fewer genotypes die annually (Eriksson 1989; Cain 1990; Watkinson & Powell 1993). Besides, a clone may live for many years and may exist of many flowering rosettes, thereby increasing the per-year-probability that a genotype is involved in successful sexual reproduction. It is also remarkable that *S. pratensis* and *H. radicata* have very similar patterns of sexual reproduction pathways (Fig. 3C,D), although they differ in longevity. Perhaps the differences in longevity are less important for the pattern of sexual reproduction than differences in clonality.

The low probabilities of seedling establishment can only be explained to a small extent by inviability of the intact seeds that were used for the seed addition experiments. Soons and Heil (2002) studied the same four species and report germination rates lower than 50% only in *S. pratensis* (20%). Other factors like microsite limitation and postdispersal seed predation are therefore probably more important for explaining these low recruitment probabilities.

Seedling recruitment in *S. pratensis* was only slightly higher in the sod-cut areas than in the meadows. Comparable establishment rates (ca 10%) were found in artificial gaps in a Czech meadow (Kotorová & Lepš 1999). Top soil and vegetation removal creates opportunities for seedlings, but also causes abiotic stress due to ammonium accumulation (de Graaf, Verbeek, Bobbink & Roelofs 1998; Dorland, Bobbink, Messelink & Verhoeven 2003). This effect has been shown to hamper the establishment of *C. dissectum* and *S. pratensis* seedlings for up to a year after sod cutting (Dorland et al. 2003). However, we started the seed addition experiment at least ten months after sod cutting. *Succisa pratensis* seems to be a relatively good establisher in closed vegetations, whereas *C. dissectum* still is expected to establish better in sod cut areas but apparently still in low numbers. *Hypochaeris radicata* and *C. jacea*, however, did benefit from vegetation removal. Their ability to utilize open spaces may be part of the explanation why these two species are still relatively common.

Flower production per flower head causes the largest increase in the number of ovules per flowering rosette. In *C. dissectum*, however, seed set is low and only weakly correlated with flower production. Jump and Woodward (2003) report lower seed set for *Cirsium acaule* and *Cirsium heterophyllum* towards the edge of their range in the UK. Perhaps similar edge and population density effects influenced *C. dissectum*, which is a very rare species in the Netherlands. Losses due to seed predation were most significant in *C. jacea* (18% of the seeds on average). In accordance with Fenner, Cresswell, Hurley & Baldwin (2002) larger flower heads of *C. jacea*, with more flowers, tended to have higher predation probabilities ($n=375$; $F=3.38$; $p=0.067$). This effect was rendered insignificant, however, when site and year differences were taken into account. A cause of destruction of buds of flower heads in *S. pratensis* is the caterpillar-like larva of the sawfly *Abia sericea* (McGee 2001), which feeds specifically on *S. pratensis* and was found at several sites. However, all these losses are less severe than the losses in the seedling establishment step.

Seed production is not only important for within-population dynamics, but also for the regional population dynamics: the number of produced seeds partly determines the colonization capacity of a species (Soons et al. 2004; Soons, Nathan & Katul 2004). When studying the effects on a species' regional survival by habitat fragmentation or habitat range displacements due to climate change, seed production and seed dispersal are therefore possibly as important as seedling establishment.

Temporal and spatial variation

The question whether populations are seed or microsite limited is much debated (Eriksson & Ehrlén 1992; Coulson, Bullock, Stevenson & Pywell 2001). Within a population, recruitment is the product of seed production and seedling establishment. A relative increase in one is as important as a relative increase in the other. However, between populations comparisons of relative limitation can be made. Comparing sites, for instance, we find that meadows are more microsite-limited than sod-cut areas in the two more common species, *C. jacea* and *H. radicata*.

The largest reductions in the number of offspring took place during seedling recruitment, but it would be premature to conclude that this is the only important bottleneck in the local sexual reproduction pathway of perennials. Rather, high within-population temporal coefficients of variation indicate where selection can have the biggest effect (Koenig, Kelly, Sork, Duncan, Elkinton et al. 2003). In our study it was again seedling establishment that had the highest within-population CVs, but temporal variability was also considerable in the number of seeds produced per flower in *C. dissectum* and the flower head production in *C. jacea* and *S. pratensis*. High spatial variation in a step of the sexual reproduction pathway may further enhance local adaptation, whereas high temporal variation may select against specialization (Bradshaw 1965; van Tienderen 1997). Therefore, the bottleneck and spatiotemporal CV analyses should be used complementary since they answer the general question of which step in reproduction is most important in a different way, although they highlight mostly the same steps in our grassland herbs.

Acknowledgements

We are grateful to Frank Berendse, Teja Tscharntke, two anonymous reviewers and the members of the Nature Conservation and Plant Ecology PhD-group for their useful comments on earlier drafts of this manuscript. Lidewij Keser assisted with the numerous flower and seed counts. The park rangers of the community of Ermelo and of the Dutch State Forestry kindly gave permission to perform this study in their meadows. The Netherlands Organization for Scientific Research funded this study (NWO-project 805.33.452).

Reference List

Berg, H. (2003). Factors influencing seed : ovule ratios and reproductive success in four cleistogamous species: A comparison between two flower types. *Plant Biology*, *5*, 194-202.

Bradshaw, A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics, 13*, 115-155.

Brys, R., Jacquemyn, H., Endels, P., Van Rossum, F., Hermy, M., Triest, L., De Bruyn, L., & De Blust, G.D.E. (2004). Reduced reproductive success in small populations of the self-incompatible *Primula vulgaris*. *Journal of Ecology*, *92*, 5-14.

Cain, M.L. (1990). Models of clonal growth in *Solidago altissima*. *Journal of Ecology*, *78*, 27-46.

Cheplick, G.P. (1995). Plasticity of seed number, mass, and allocation in clones of the perennial grass *Amphibromus scabrivalvis*. *International Journal of Plant Sciences*, *156*, 522-529.

Coulson, S.J., Bullock, J.M., Stevenson, M.J., & Pywell, R.F. (2001). Colonization of grassland by sown species: dispersal versus microsite limitation in responses to management. *Journal of Applied Ecology*, *38*, 204-216.

Crawley, M.J. (1997). Plant Ecology. Oxford: Blackwell Science.

de Graaf, M.C.C., Verbeek, P.J.M., Bobbink, R., & Roelofs, J.G.M. (1998). Restoration of species-rich dry heaths: The importance of appropriate soil conditions. *Acta Botanica Neerlandica*, *47*, 89-111.

de Kroon, H., van Groenendael, J.M., & Ehrlén, J. (2000). Elasticities: a review of methods and model limitations. *Ecology*, *81*, 607-618.

Dorland, E., Bobbink, R., Messelink, J.H., & Verhoeven, J.T.A. (2003). Soil ammonium accumulation after sod cutting hampers the restoration of degraded wet heathlands. *Journal of Applied Ecology*, *40*, 804-814.
Eriksson, O. (1989). Seedling dynamics and life histories in clonal plants. *Oikos*, *55*, 231-238.
Eriksson, O., & Ehrlén, J. (1992). Seed and microsite limitation of recruitment in plant populations. *Oecologia*, *91*, 360-364.
Fenner, M., Cresswell, J.E., Hurley, R.A., & Baldwin, T. (2002). Relationship between capitulum size and pre-dispersal seed predation by insect larvae in common Asteraceae. *Oecologia*, *130*, 72-77.
Fowler, J, Cohen, L., & Jarvis, P. (1998). Practical statistics for field biology. Chichester: John Wiley & sons.
Goodman, L.A. (1960). On the exact variance of products. *Journal of the American Statistical Association*, *55*, 708-713.
Harper, J.L. (1977). Population biology of plants. London: Academic Press.
Hartemink, N., Jongejans, E., & de Kroon, H. (2004). Flexible life history responses to flower and rosette bud removal in three perennial herbs. *Oikos*, *105*, 159-167.
Herrera, C.M. (2000). Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub. *Ecology*, *81*, 15-29.
Isselstein, J., Tallowin, J.R.B., & Smith, R.E.N. (2002). Factors affecting seed germination and seedling establishment of fen-meadow species. *Restoration Ecology*, *10*, 173-184.
Jakobsson, A., & Eriksson, O. (2000). A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos*, *88*, 494-502.
Jensen, K., & Gutekunst, K. (2003). Effects of litter on establishment of grassland plant species: the role of seed size and successional status. *Basic and Applied Ecology*, *4*, 579-587.
Jongejans, E., & de Kroon, H. (2005). Space versus time variation in the population dynamics of three co-occurring perennial herbs. *Journal of Ecology*, *93*, doi: 10.1046/j.0022-0477.2005.01003.x.
Jongejans, E., & Schippers, P. (1999). Modeling seed dispersal by wind in herbaceous species. *Oikos*, *87*, 362-372.
Jump, A.S., & Woodward, E.I. (2003). Seed production and population density decline approaching the range-edge of *Cirsium* species. *New Phytologist*, *160*, 349-358.
Klinkhamer, P.G.L., de Jong, T.J., & van der Meijden, E. (1988). Production, dispersal and predation of seeds in the biennial *Cirsium vulgare*. *Journal of Ecology*, *76*, 403-414.
Koenig, W.D., Kelly, D., Sork, V.L., Duncan, R.P., Elkinton, J.S., Peltonen, M.S., & Westfall, R.D. (2003). Dissecting components of population-level variation in seed production and the evolution of masting behavior. *Oikos*, *102*, 581-591.
Kotorová, I., & Lepš, J. (1999). Comparative ecology of seedling recruitment in an oligotrophic wet meadow. *Journal of Vegetation Science*, *10*, 175-186.
Lamont, B.B., & Runciman, H.V. (1993). Fire may stimulate flowering, branching, seed production and seedling establishment in two Kangaroo paws (Haemodoraceae). *Journal of Applied Ecology*, *30*, 256-264.

Lienert, J., & Fischer, M. (2004). Experimental inbreeding reduces seed production and germination independent of fragmentation of populations of *Swertia perennis*. *Basic and Applied Ecology, 5*, 43-52.

Louda, S.M., & Potvin, M.A. (1995). Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. *Ecology*, *76*, 229-245.

McGee, K. (2001). *Abia* Sawflies at Mill Meadow, Drakes Broughton. *Worcestershire Record*, *6*.

Menges, E.S. (1995). Factors limiting fecundity and germination in small populations of *Silene regia* (Caryophyllaceae), a rare hummingbird-pollinated prairie forb. *American Midland Naturalist*, *133*, 242-255.

Meyer, A.H., & Schmid, B. (1999). Seed dynamics and seedling establishment in the invading perennial *Solidago altissima* under different experimental treatments. *Journal of Ecology*, *87*, 28-41.

Mix, C., Picó, F.X., & Ouborg, N.J. (2003). A comparison of stereomicroscope and image analysis for quantifying fruit traits. *Seed Technology*, *25*, 12-19.

Olson, B.E., & Wallander, R.T. (2002). Effects of invasive forb litter on seed germination, seedling growth and survival. *Basic and Applied Ecology, 3*, 309-317.

Oostermeijer, J.G.B., Luijten, S.H., Krenová, Z.V., & den Nijs, H.C.M. (1998). Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. *Conservation Biology*, *12*, 1042-1053.

Picó, F.X., & Riba, M. (2002). Regional-scale demography of *Ramonda myconi*: Remnant population dynamics in a preglacial relict species. *Plant Ecology*, *161*, 1-13.

Schippers, P., & Jongejans, E. (2005). Release thresholds strongly determine the range of seed dispersal by wind. *Ecological Modelling*, *184*, doi: 10.1016/j.ecolmodel.2004.11.018.

Schupp, E.W. (1990). Annual variation in seedfall, postdispersal predation, and recruitment of a Neotropical tree. *Ecology*, *71*, 504-515.

Silvertown, J., Franco, M., Pisanty, I., & Mendoza, A. (1993). Comparative plant demography: Relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. *Journal of Ecology*, *81*, 465-476.

Soons, M.B., & Heil, G.W. (2002). Reduced colonization capacity in fragmented populations of wind-dispersed grassland forbs. *Journal of Ecology*, *90*, 1033-1043.

Soons, M.B., Heil, G.W., Nathan, R. & Katul, G.G. (2004). Determinants of long-distance seed dispersal by wind in grasslands. *Ecology*, *85*, 3056-3068.

Soons, M.B., Messelink, J.H., Jongejans, E., & Heil, G.W. (2003). Fragmentation and connectivity of species-rich semi-natural grasslands. In: M.B. Soons (Ed.), *Habitat fragmentation and connectivity. Spatial and temporal characteristics of the colonization process in plants.* Ph.D. thesis, Utrecht University.

Soons, M.B., Nathan, R., & Katul, G.G. (2004). Human effects on long-distance wind dispersal and colonization by grassland plants. *Ecology*, *85*, 3069-3079.

Špačková, I., & Lepš, J. (2004). Variability of seedling recruitment under dominant, mos, and litter removal over four years. *Folia Geobotanica*, *39*, 41-55.

Szentesi, A., & Jermy, T. (2003). Pre-dispersal seed predation and seed limitation in an annual legume. *Basic and Applied Ecology*, *4*, 207-218.

Thompson, K., Bakker, J.P., & Bekker, R.M. (1997). The soil seed banks of North West Europe: methodology, density and longevity. Cambridge: University Press.

van Tienderen, P.H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution, 51*, 1372-1380.

Watkinson, A.R., & Powell, J.C. (1993). Seedling recruitment and the maintenance of clonal diversity in plant populations: A computer simulation of *Ranunculus repens*. *Journal of Ecology*, *81*, 707-717.

Table 1.

Statistics of type III ANOVA models per species. Where reported seed addition and field type are built in as fixed factors, year and site as random factors, and flower number and predation (absent or present) as covariates. To increase homogeneity among the data, the number of flower heads were arcsinh-transformed, the number of seeds produced per flower arcsine-transformed, and the increases in rosette number were log-transformed after adding 10 (Fowler, Cohen & Jarvis 1998). Back transformed site and year means are presented in Table 2 and Figure 2. (*) = p<0.1; *=p<0.05; **=p<0.01;***=p<0.001

	Centaurea jacea			*Cirsium dissectum*			*Hypochaeris radicata*			*Succisa pratensis*		
Source	df	MS	F	df	MS	F	df	MS	F	df	MS	F
# Flower heads per Flowering Rosette												
Site (S)	2	10.573	13.60 **	2	0.276	0.75	2	1.210	2.05	4	10.631	1.67
Year (Y)	4	5.816	7.92 *	4	0.244	0.63	4	1.025	1.77	4	13.080	2.58 (*)
S * Y	6	0.761	2.72 *	8	0.439	6.78 ***	7	0.755	2.25 *	15	7.578	15.77 ***
Error	622	0.280		267	0.065		207	0.336		1162	0.470	
# Flowers per Flower head												
Site (S)	4	12672	26.55 ***	9	2395	1.94	5	7209	5.01 (*)	12	7112	7.59 ***
Year (Y)	4	697	1.34	4	12475	8.57 **	3	4900	3.66 (*)	4	1126	1.75
S * Y	8	531	3.29 **	14	1526	4.04 ***	5	1421	3.07 *	24	1036	7.44 ***
Error	358	161		685	378		293	462		794	139	
Ratio #Seeds / #Flowers												
Flower number	1	891	4.05 *	1	485	3.04 (*)	1	3328	23.16 ***	1	797	4.22 *
Predation	1	36071	163.99 ***	1	8180	51.34 ***	1	438	3.05 (*)	1	1468	7.77 **
Site (S)	4	4087	6.68 **	9	5346	8.35 ***	5	1020	6.40 *	12	3062	2.79 *
Year (Y)	4	220	0.30	4	5726	7.73 **	3	327	2.06	4	978	1.24
S * Y	8	749	3.41 **	14	813	5.10 ***	5	160	1.11	24	1253	6.63 ***
Error	356	220		683	159		291	144		792	189	
Increase Number rosettes (Seed addition Experiment 1)												
Seed Addition	1	0.002	0.25	1	0.001	0.19	1	0.111	4.24 *	1	0.134	16.47 ***
Site (S)	2	0.045	0.90	1	0.156	348.58 *	1	-	-	1	0.002	0.02
Year (Y)	1	0.026	0.53	1	0.003	6.19	1	-	-	1	0.000	0.00
S * Y	1	0.049	5.27 *	1	0.000	0.14	0	-	-	1	0.097	11.88 **
Error	94	0.009		75	0.003		56	0.026		75	0.008	
Increase Number rosettes (Seed addition Experiment 2)												
Seed Addition (SA)	1	0.156	37.15 ***	1	0.009	9.45 **	1	0.764	92.51 ***	1	0.655	71.92 ***
Site within T	8	0.022	5.32 ***	8	0.004	4.75 ***	8	0.046	5.52 ***	8	0.076	8.39 ***
Type (T)	1	0.004	5.96 *	1	0.019	4.28 (*)	1	0.370	8.12 *	1	0.027	0.36
SA * T	1	0.127	30.16 ***	1	0.004	4.58 *	1	0.162	19.68 ***	1	0.034	3.77 (*)
Error	148	0.004		148	0.001		148	0.008		148	0.009	

Table 2.

The overall mean, and the range (minimum and maximum) of the site and year means separately, of the number of flower heads per flowering rosette, flowers per flower head and seeds per flower. The number of sites, years and site-year combinations that were sampled for each species, and the mean number of samples per site-year combination are also given. The mean number of seeds per flower in predated flower heads was only calculated for a site-year combination when predated heads were present. The means of the number of flower heads and seeds presented here are back transformed means of transformed data (Table 1) on head (arcsinh) and seed (arcsine) numbers.

		Overall	Site (S)		Year (Y)		SxY	# Samples
Variable	Species	Mean	n	Range	n	Range	n	Mean
# Flower heads per Flowering rosette								
	Centaurea jacea	1.31	3	1.04 - 1.83	5	0.73 - 1.95	13	49
	Cirsium dissectum	0.87	3	0.79 - 0.97	5	0.66 - 1.00	15	19
	Hypochaeris radicata	2.20	3	2.00 - 2.45	5	1.42 - 2.48	14	16
	Succisa pratensis	2.26	5	1.45 - 3.89	5	1.11 - 3.60	24	49
# Flowers per Flower head								
	Centaurea jacea	65	5	46 - 79	5	61 - 78	17	22
	Cirsium dissectum	80	10	72 - 94	5	66 - 91	28	25
	Hypochaeris radicata	61	6	50 - 73	4	53 - 65	14	22
	Succisa pratensis	63	13	45 - 91	5	51 - 68	41	20
# Seeds per Flower (unpredated heads)								
	Centaurea jacea	0.60	5	0.00 - 0.67	5	0.48 - 0.72	17	14
	Cirsium dissectum	0.23	10	0.09 - 0.49	5	0.11 - 0.38	28	23
	Hypochaeris radicata	0.85	6	0.68 - 0.90	4	0.80 - 0.90	14	16
	Succisa pratensis	0.81	13	0.62 - 0.94	5	0.74 - 0.83	41	20
# Seeds per Flower (predated heads)								
	Centaurea jacea	0.26	5	0.00 - 0.35	5	0.21 - 0.29	17	8
	Cirsium dissectum	0.05	9	0.00 - 0.37	5	0.00 - 0.16	18	3
	Hypochaeris radicata	0.73	6	0.65 - 0.82	4	0.64 - 0.82	12	7
	Succisa pratensis	0.72	5	0.33 - 0.80	3	0.68 - 0.75	7	2

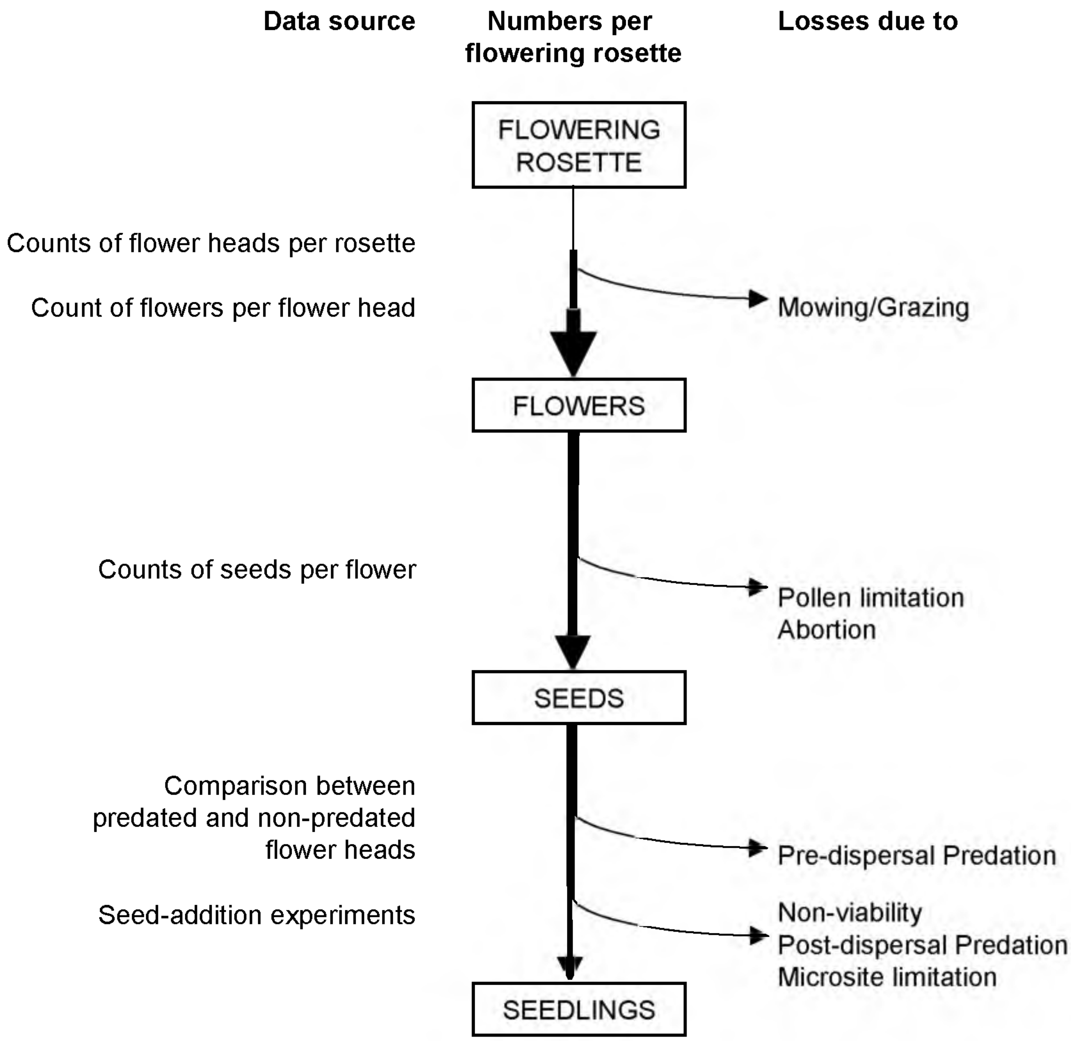

Figure 1.
Schematic overview of the sexual reproduction pathway from a flower rosette to flowers, seeds and established seedlings. Causes of losses (right) and data sources in this study on the different parts of the pathway (left) are indicated.

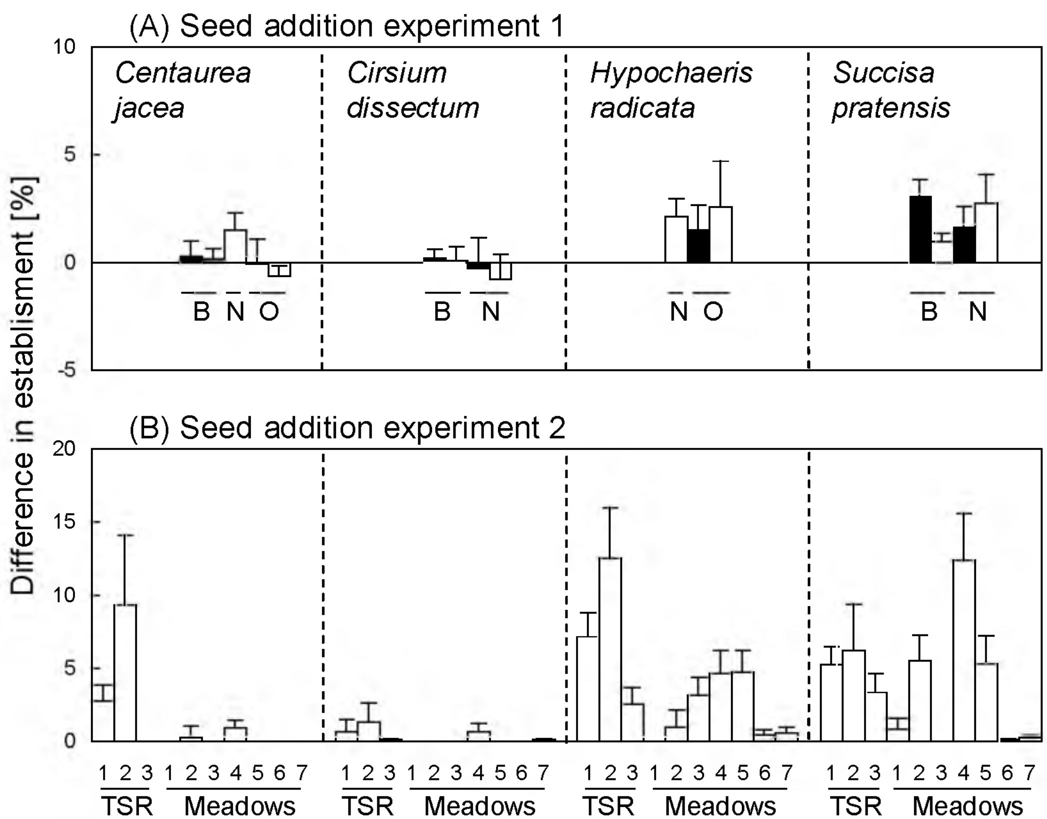

Figure 2.

The average percentage of sown seeds that is established as seedling after 12 months per species, site and year. The values (back transformed means after log transformation of the data for statistical tests; Table 1) given are the means of seeded plots minus the natural germination in the control plots. Error bars denote standard errors. The first experiment in the meadows Bennekomse meent (B), Konijnendijk (N) and Koolmansdijk (O) was started in 1999 (black) and 2000 (white). Since no local seeds were available *C. jacea* and *H. radicata* were not seeded in site N in 1999. In the second experiment seeds were sown (only in 2000) in three meadows with the top soil removed (TSR), and in seven undisturbed meadows. Please note that negative values can occur when the increase in rosette number was higher in the control plots than in the seed addition plots.

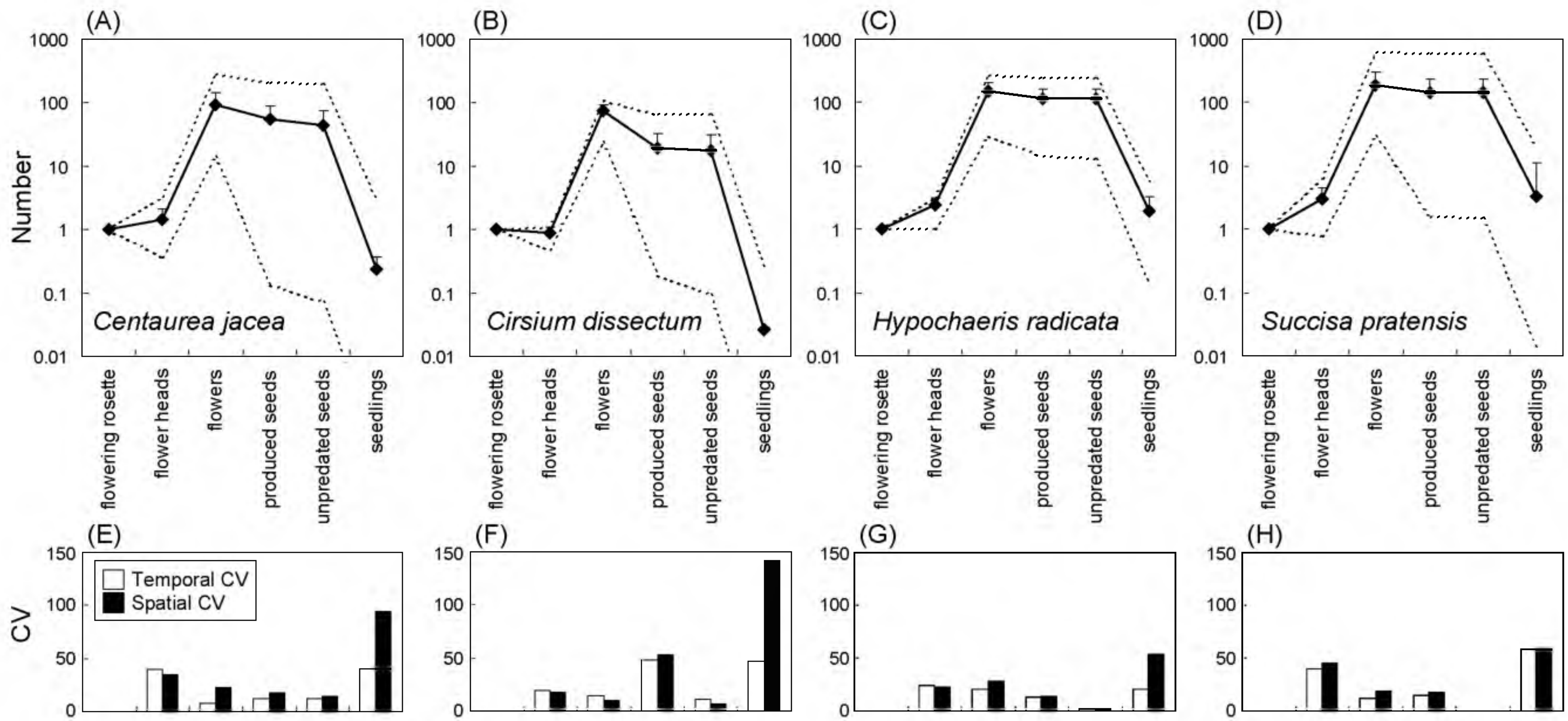

Figure 3.

The average (solid line) sexual reproduction pathway (A,B,C,D) per species, based on the means of all available site-year combinations. The error bars are progressing standard deviations (see methods). The dashed lines represent scenarios in which all steps are either the lowest or the highest observed site-year mean. The low scenario in *C. jacea* and *C. dissectum* end with zero seedlings. The spatial and temporal coefficient of variation of each step (E,F,G,H) is given per species (see methods).