

Life history strategies and biomass allocation

the population dynamics of perennial plants in a regional perspective

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General introduction

Plant populations in a changing landscape

Human impact on ecosystems threatens the survival of many species world-wide: their habitats are destroyed or degraded and become more fragmented (Tilman et al. 1994; Hanski & Ovaskainen 2000). Habitat is lost to agriculture, infrastructure and urban areas to satisfy increasing human demands. The remaining habitat patches are often small and isolated, which increases the vulnerability to negative influences from surrounding areas: disturbance, pollution through air or water, changes in water level regime, and, especially in areas of intensive agriculture, influx of nutrients (Saunders et al. 1991; Roem & Berendse 2000). Nutrient enrichment increases the productivity of nutrient-poor ecosystems and more competitive species take over from species that are characteristic for these ecosystems (de Kroon & Bobbink 1997). Besides, species richness can be expected to decrease with productivity in areas that currently receive high levels of nitrogen deposition such as the Netherlands (van Oene et al. 1999).

Large population sizes usually buffer environmental, genetic and demographic stochasticity, but the risk of extinction caused by these sources of year-to-year variation increases when populations are reduced to low numbers of individuals by habitat fragmentation and environmental factors (Menges 1998; Oostermeijer 2003). Small populations contain less genetic variation that may be necessary to adapt to changing environmental conditions (Ellstrand & Elam 1993). Demographic stochasticity in population dynamics is for instance the chance event of simultaneously a high death rate and a low establishment rate in one year. Besides, small populations may perform less just because they are small (e.g. because they fail to effectively attract pollinators), which is called the Allee-effect (Fischer & Matthies 1998; Colas et al. 2001), or because inbreeding starts to affect the performance of individuals.

The increasing distances between populations amplifies these problems, as they diminish the probabilities of arrival of genetically-different seeds or pollen in areas with populations that are decreasing in size or are already extinct. Genetically impoverished populations are therefore unlikely to survive. Metapopulation theory predicts that the number of occupied habitats declines with habitat size and habitat connectivity (MacArthur & Wilson 1967; Ouborg 1993). Climate change increases the importance of connectivity as species have to disperse to keep up with possible change in the spatial distribution of climates they are adapted to (Thomas et al. 2004).

Since most nations engaged the obligation to protect our plant species at the Rio convention on biological diversity (Myers 1993), the above-mentioned issues urge to answer the important question: can plant species survive in highly-fragmented landscapes such as we find in the Netherlands?

The research program 'Survival of plant species in fragmented landscapes'

To generate data and knowledge on the mechanisms that affect the survival chances of plant species in fragmented landscapes, the Netherlands Organization for Scientific Research (NWO-ALW) financed the research program 'Survival of plant species in fragmented landscapes'. In this program, which was chaired by Jan van Groenendael, plant ecologists from several institutes worked together on different aspects of the topic in the same study system: nutrient-poor, species-rich meadows. Three projects were started on the landscape ecology, local population dynamics, and genetics of a set of model species respectively. The projects were integrated by close cooperation by the PhD students involved and by a post-doctoral modelling project.

At Utrecht University Merel Soons finished her PhD thesis in 2003: 'Habitat fragmentation and connectivity. Spatial and temporal characteristics of the colonization process in plants'. Her research focused on the effects of habitat fragmentation on the dispersal of seeds between habitat patches and the colonization abilities of plants in realistic landscapes.

'Life history strategies and biomass allocation. The populations dynamics of perennial plants in a regional perspective' is the title of the second project, that resulted in the PhD thesis in front of you. At Wageningen University I studied the dynamics of natural plant populations, and the impact of nutrient enrichment on life history components of these plants in experiments and population models.

At the University of Nijmegen Carolin Mix performed a PhD study entitled 'Inbreeding and outbreeding: effects of gene flow and local adaptation on the survival of small isolated populations of plants in a regional context'. She investigated the genetic effects of habitat fragmentation on the performance of the remaining populations, which are often small and isolated.

Felix Knauer used model exercises and the results of these three projects to study the effectiveness of ecological corridors and agri-environmental schemes, which aim to alleviate the isolation of remnant populations. The title of his post-doctoral project at Alterra (Wageningen UR) was 'Integration and application: regional survival in changing landscapes'.

Study system: herb species of nutrient-poor, species-rich meadows

The studied habitats were restricted to nutrient-poor grassland fragments of the Pleistocene soil areas of the Netherlands (Soons 2003), which include the plant communities *Molinietalia* and *Caricetalia* (Schaminée et al. 1995; Schaminée et al. 1996). These moist grasslands were formerly used as hay meadows and increased in abundance in the 19th century when an increasing area of peat bogs and fens was cultivated (Buck-Sorlin 1993). In the 20th century (especially the first half) more than 99% of these habitats was lost (Soons 2003), probably due to intensification of cultivation for agriculture by drainage and fertilization (Buck-Sorlin 1993). Most of the remnants of these semi-natural grasslands have now become nature reserves and are

managed by continuing the old land use practices: mowing and hay removal, in order to counter 1) the encroachment of shrubs and trees, and 2) the disrupting effect of nutrient enrichment (Bakker & Berendse 1999).

As model species we chose four species that occur in these meadows and that are phylogenetically closely related (all belong to the families Asteraceae or Dipsacaceae). They have the same life history options (i.e. sexual reproduction, survival and clonal propagation; cf. Fig. 1), but they have distinctly different life history strategies, i.e. they differ in longevity and clonality: *Hypochaeris radicata* L. plants normally live only for a few years, whereas *Succisa pratensis* Moench. (through

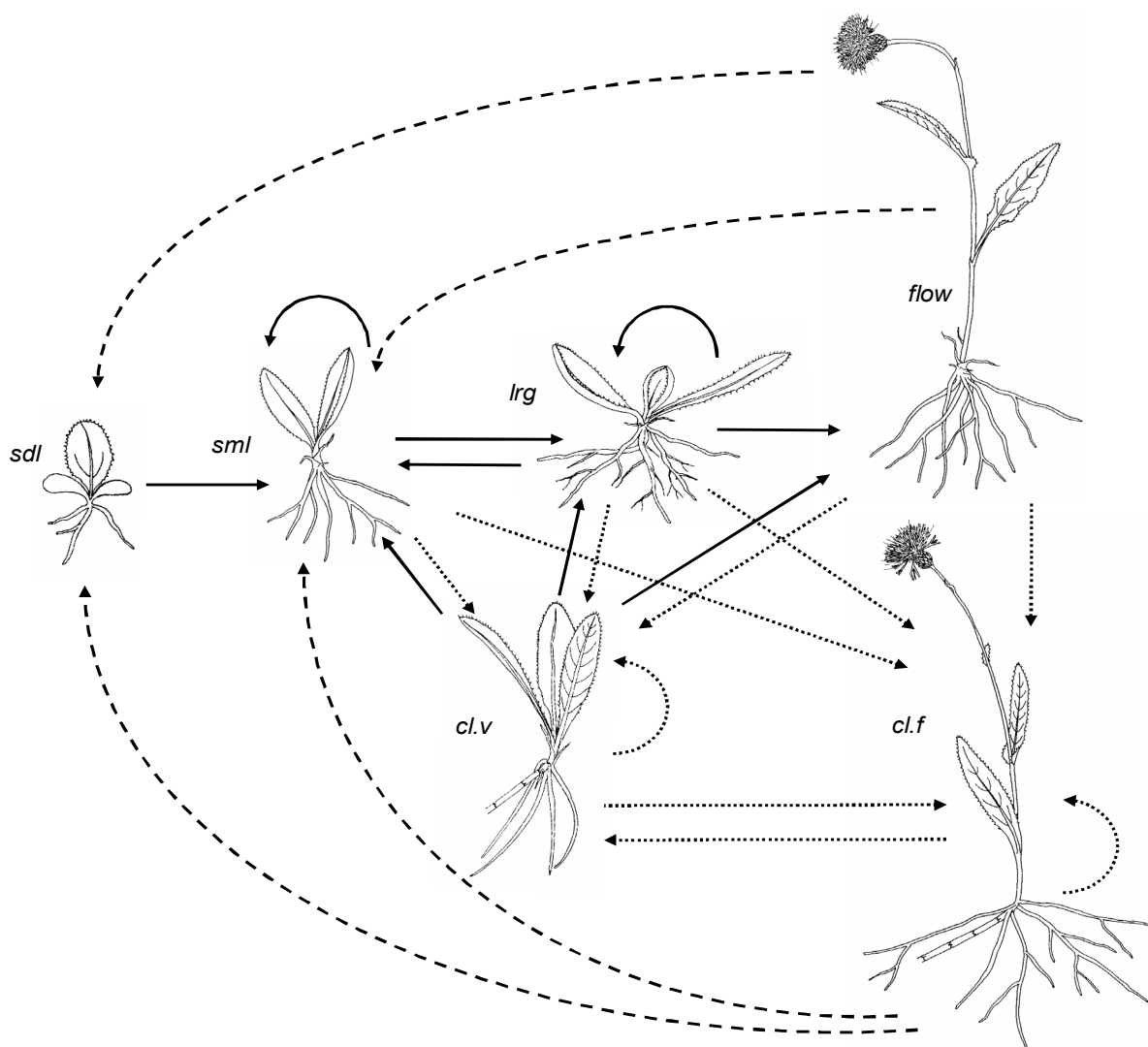


Figure 1.

The life cycle of *Cirsium dissectum* with six stage classes: seedlings (*sdl*), small vegetative rosettes (*sml*), large vegetative rosettes (*lrg*), flowering rosettes (*flow*), vegetative clonal offspring (*cl.v*), and flowering clonal offspring (*cl.f*). The arcs denote the contribution probabilities of plants of one stage class to a particular stage class one year later. Transitions can be grouped as survival (solid lines), sexual reproduction (interrupted lines), or clonal propagation (dotted lines). The different rosettes are not depicted on the same scale of size. The drawings were made by Lidewij Keser.

rosette survival), *Centaurea jacea* L. and *Cirsium dissectum* (L.) Hill (clonal propagation) can potentially survive for decades. The term 'life history strategy' can be defined as the complex adaptation of all aspects of the life cycle of a species (Stearns 1992). Important factors to which species can adapt are for instance harsh abiotic conditions, unfavorable seasons, competition and herbivory (Grime 1979). The life cycle of perennial plant species consists of several alternative pathways which can be grouped as survival, sexual reproduction and clonal propagation (the life cycle of *C. dissectum* is given as an example in Fig. 1).

The model species were also selected because they not only differ in longevity but also in dispersal ability (Ehrlén & van Groenendael 1998). The seeds of *H. radicata* and *C. dissectum* have pappus, which enables them to be carried further by wind (Soons & Heil 2002; Mix et al. 2003). The other two species have no adaptation for dispersal by wind. Besides, *C. dissectum* and *S. pratensis* are mostly restricted to the above-defined habitats, whereas *C. jacea* and *H. radicata* also occur in more productive or more disturbed areas (van der Meijden 1996). The distances between populations are therefore larger in the first two species (Soons 2003).

Life history components, trade-offs and the effect of nutrient enrichment

Since life history components are not independent of each other, research on the life history of species should also consider their interrelations. Resources that are invested in one component, e.g. sexual reproduction, are in theory no longer available for other components, e.g. growth or clonal propagation. Physiological trade-offs between life history components can be expected based on this theory (Stearns 1992). In natural conditions however, trade-offs are not readily found. There are several explanations why often positive instead of negative correlations are observed: because flowering stems bearing green leaves can be self supporting (Benner & Watson 1989) or because high variability in growing conditions between plants masks trade-offs. But the most important one is that most life history components are dependent on plant size (van Noordwijk & de Jong 1986; Reznick et al. 2000): large plants have more to spend on all components compared to small plants (Fig. 2). The fact that costs of life history components are not apparent does not mean they do not exist. Manipulative experiments that prohibit investment in a particular life history component can show a negative impact of one component on other components (Stearns 1992; Ehrlén 1999). The flexibility of plants in their responses to manipulations needs to be considered in such experiments, as the reallocation of resources and the activation of meristems is often time-limited (Watson 1984; Geber 1990).

Effects of nutrient enrichment have been found in the model species: Soons and Heil (2002) reported higher seed production in more nutrient-rich sites. The allocation patterns, i.e. the relative investment of resources in different life history components, are also hypothesized to respond to changes in growing conditions due to nutrient enrichment and succession of the vegetation (Abrahamson 1980).

Specifically, plants are thought to have two possibilities when the productivity of a vegetation increases: 1) to escape to other sites through seed dispersal, or 2) to persist by reinforcing their competitive position (Ogden 1974; Abrahamson 1980). Although these long-standing hypotheses have clear expectations of respectively increased and decreased resource allocation to seed production, it has never been explicitly tested in experiments.

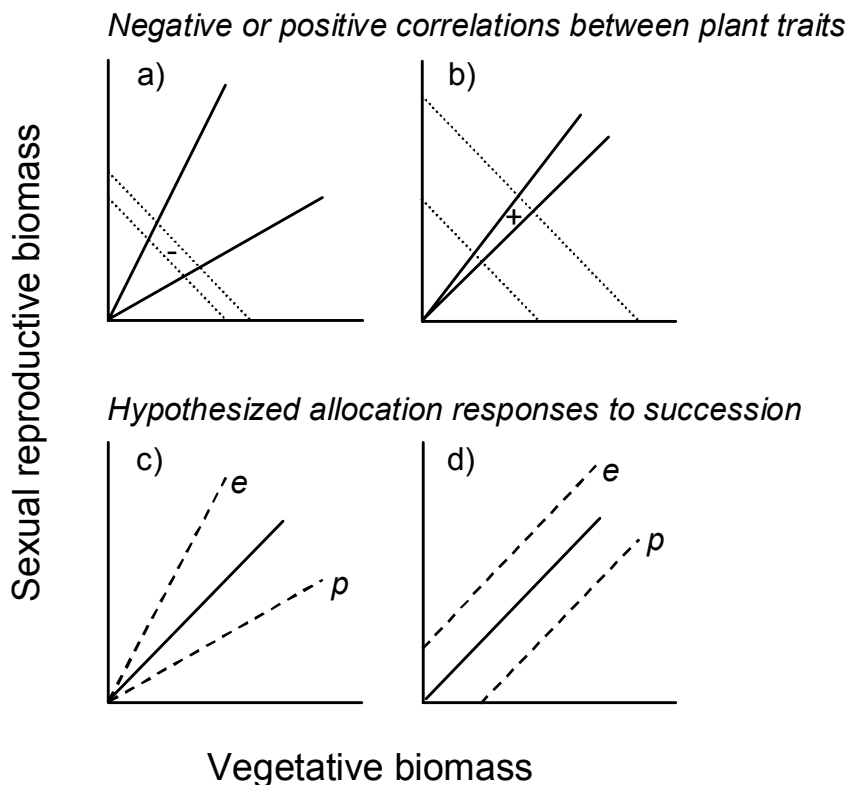


Figure 2.

A summary of two important allocation theories used in this dissertation: the hypothesis that the type of correlation between plant traits depends on the variation in plant size and the variation in allocation, and the hypothesized plant responses to succession. In these schematic models of allocation patterns solid lines symbolize the proportion of biomass plants invest in either sexual reproductive biomass (flowers and seeds) as a function of vegetative biomass (roots, leaves and stems). The upper diagrams show how the observed correlation between the sexual and vegetative biomass depends on variation in allocation pattern and on variation in plant size (distance between dotted lines) (van Noordwijk & de Jong 1986): a) negative correlations are found when plant size varies little, but variation in allocation patterns is large, b) positive correlations are found when allocation patterns differ less between plants than the variation in their sizes. The bottom diagrams show how the hypothesized escape (e) and persistence (p) strategies (Abrahamson 1980) alter the allocation patterns. Sugiyama and Bazzaz (1998) show that sexual reproductive allocation can be changed c) relatively to vegetative size or d) by an absolute amount independent of vegetative plant size.

Table 1.

Presence and abundance of higher plant species in the permanent plots used for the demographic study on the four species at the left top (studied species-site combinations in bold). The area of all plots for each meadow was 6 m² in Konijnendijk (N), 4 m² in Koolmansdijk (O), 5 m² in Bennekomse meent (B), 8 m² in Leemputten (L), and 3 and 2 m² respectively for the two locations in Veerslootlanden (V1 and V2). Species lists were made in July and August, 1999 and 2000. Nomenclature follows van der Meijden (1996). For each species present in a site the median abundance of the plots in which it is present is given. Braun-Blanquet abundance codes are used: r = 1 specimen; + = 2-5 specimens; 1 = 6-50 specimens; 2m = >50 specimens, but cover is still <5%; 2a = 5-12% cover; 2b = 13-25% cover; 3 = 25-50% cover; 4 = 50-75% cover; 5 = 75-100% cover.

Species	N	O	B	L	V1	V2	Species	N	O	B	L	V1	V2
<i>Centaurea jacea</i>	1	1	1		r	2a	<i>Juncus conglomeratus</i>	+		1		1	1
<i>Cirsium dissectum</i>	3		2a			3	<i>Leontodon autumnalis</i>		r				
<i>Hypochaeris radicata</i>	1	1		+			<i>Leucanthemum vulgare</i>		+				
<i>Succisa pratensis</i>	+	1	1	2a	1		<i>Linum catharticum</i>				+		
<i>Achillea millefolium</i>		+					<i>Luzula campestris</i>	+	+			+	
<i>Agrostis</i> spp	2a	1	1	1			<i>Luzula multiflora</i>			+			
<i>Anthoxanthum odoratum</i>	+	1	1			r	<i>Lysimachia vulgaris</i>	1	r				
<i>Betula pendula</i>				+			<i>Lythrum salicaria</i>	r					
<i>Betula pubescens</i>	1	1					<i>Molinia caerulea</i>	2b	+	2b	2a	5	+
<i>Calluna vulgaris</i>	1			1			<i>Parnassia palustris</i>				r		
<i>Carex flacca</i>				1			<i>Pedicularis sylvatica</i>				+		
<i>Carex nigra</i>	1		2a				<i>Peucedanum palustre</i>						r
<i>Carex oederi</i>				r			<i>Phragmites australis</i>			2a			1
<i>Carex panicea</i>	1	2b	2a	1	1	2b	<i>Pinus sylvestris</i>				r		
<i>Carex pulicaris</i>				1			<i>Plantago lanceolata</i>		2a	1			
<i>Carlina vulgaris</i>				r			<i>Polygala serpyllifolia</i>				1		
<i>Cirsium palustre</i>			r				<i>Potentilla erecta</i>	2a	+	2m	+	1	+
<i>Dactylorhiza maculata</i>	+	+		r			<i>Prunella vulgaris</i>	+		r	+		r
<i>Danthonia decumbens</i>	+	1	+	1	+	+	<i>Quercus robur</i>	+	r				
<i>Drosera rotundifolia</i>				r			<i>Ranunculus acris</i>	r					
<i>Equisetum arvense</i>		r					<i>Ranunculus flammula</i>						r
<i>Equisetum palustre</i>			1				<i>Rhamnus frangula</i>	+			r		
<i>Erica tetralix</i>	x			2a			<i>Rhinanthus angustifolius</i>			+	+		
<i>Euphrasia stricta</i>				1			<i>Rumex acetosa</i>		r				
<i>Festuca</i> spp	1	1	2b	1	2a	2b	<i>Salix cinerea</i>		+		1		
<i>Filipendula ulmaria</i>			+			+	<i>Salix repens</i>	+	+				
<i>Gentiana pneumonanthe</i>	+		r	+			<i>Sanguisorba officinalis</i>					+	1
<i>Hieracium laevigatum</i>	+	1					<i>Sorbus aucuparia</i>	r					
<i>Holcus lanatus</i>		+					<i>Taraxacum officinale</i>	r					
<i>Hypericum perforatum</i>		+					<i>Thalictrum flavum</i>						1
<i>Hypericum pulchrum</i>				+			<i>Trichophorum cespitosum</i>				r		
							<i>Viola arvensis</i>			+			+

Population dynamics of perennial plant species

Detailed data on the life cycle of plant species under natural conditions is needed to assess the importance of particular life history components, and of the factors affecting them. Simple demographic observations, i.e. monitoring the fate of individual plants from year to year and the rate at which seedlings and clonal offspring establish, can provide a wealth of information. Demographic stochasticity, one of the factors that determines extinction risks of small populations, can be quantified when multiple

populations are followed over several years (Oostermeijer et al. 1996). In general, survival becomes more important at the expense of growth and fecundity when growing conditions are less favorable (Oostermeijer et al. 1996; Menges & Dolan 1998). Whether temporal and spatial variation in population dynamics are equal or not (whether plants similarly respond to good years as to good sites) is largely unknown and needs more investigation.

Population matrix models are very suitable for analyzing demographic field data (Caswell 2001). In plants mostly Lefkovitch matrices are used. These classify plants on their phenological stage and their size rather than on age (Lefkovitch 1965). These transition matrices can be analyzed in different ways: elasticity analysis calculates the relative importance of each matrix element (i.e. the arcs in Fig.1) for the growth rate of the population (de Kroon et al. 1986). Elasticities allow comparison between very dissimilar species or growing conditions due to their proportional nature (Silvertown et al. 1993). However, analyzing what differences in life history components contributed to observed differences in population growth rate requires variation decomposition techniques (Horvitz et al. 1997; Caswell 2001). Moreover, the direct and indirect importance of underlying plant traits can be studied with hierarchical matrix models (van Tienderen 2000). Issues concerning the impact of those factors on the population dynamics however can only be addressed after the quantification of the natural population dynamics and of the effects of external factors on specific life history components.

Outline of this thesis

In this dissertation I study the allocation patterns and population dynamics of the four model species and the effect of increased productivity in experiments and modeling exercises. Specifically, in chapter 2 the costs of sexual reproduction and clonal propagation are investigated by continuously removing flower buds and rosette buds. Apart from the altered biomass allocation patterns, we study the flexibility of the responses to bud removal in this one-season garden experiment.

In chapter 3 we test the hypothesis that individual plants can alter their allocation patterns when the biomass and height of the surrounding vegetation increases. In the same garden experiment as reported in the previous chapter, but now for three years, we added nutrients to plots with the studied species and tussocks of a tall grass, and we investigate how plants change their biomass allocation compared to unfertilized control plants.

Chapter 4 deals with the variation in the sexual reproduction pathway within and between species. We try to answer the question which step in the sexual reproduction pathway is the largest bottleneck from flowering adult to established seedling. We compare the survival probabilities in the different steps with the variability of those steps to see whether these two analyses pinpoint different steps as being most important.

Whether temporal variation can be substituted with spatial variation in the

population dynamics of perennial plant species is the central question of chapter 5. We studied the population dynamics of three of the model species in five sites (Table 1) and use matrix projection models and variation decomposition analyses to examine whether the life histories of these species respond similarly to bad sites as to bad years.

In chapter 6 measured levels of inbreeding depression are incorporated in population projection matrices. The impact of inbreeding depression and density dependency of the population dynamics are studied in a long-lived herb *Succisa pratensis* with stochastic simulation modeling.

Chapter 7 investigates the population dynamics of the endangered, clonal herb *Cirsium dissectum*, which propagates through rhizomes. Special attention is paid to whether the allocation patterns found in the third chapter are also found in natural populations over a gradient of productivity. We used variation decomposition analyses to quantify the temporal variation in population growth rate, and to see whether variation in the same life history components underlies temporal variation in the population growth rate in different populations.

In the final chapter, chapter 8, we summarize the flexibility and natural variation of the life histories of the studied species. We present an exercise with hierarchical matrix models to study which life history components and plant traits have the largest effect on variation in the population growth rate by incorporating the effects of nutrient enrichment on plant traits and life history components.

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Flexible life history responses to flower and rosette bud removal in three perennial herbs

Nienke Hartemink, Eelke Jongejans & Hans de Kroon 2004. *Oikos* 105:159-167.

Summary

In a garden experiment we investigated the response to continuous removal of either flower buds or rosette buds in three perennial grassland species (*Hypochaeris radicata*, *Succisa pratensis* and *Centaurea jacea*), which differ in longevity and flowering type. We distinguished two possible responses: compensation for lost buds by making more buds of the same type, and switching towards development of other life history functions. Both responses were demonstrated in our experiment, but bud removal had significantly different effects in each of the three species. The degree of compensation and the expression of trade-offs between life history functions differed markedly between species and seem related to longevity and developmental constraints. With respect to switching, our results suggest costs of reproduction and a trade-off between life history functions, at least for *H. radicata* and *S. pratensis*. For these species weight of new rosettes increased when resource allocation to flowering was inhibited. In *H. radicata*, we see that both compensation for lost flower buds and switching from lost rosette buds increased production of flower buds, underscoring the pivotal role of sexual reproduction in this short-lived species. The most prominent response seen in *C. jacea* is compensation for lost rosette buds, indicating that this long-lived species with monocarpic rosettes relies on rosette formation. Although *S. pratensis* does respond to bud removal, time is an important constraint in this species with long-lived rosettes and preformed flowering stalks. Trade-offs in *S. pratensis* seem to operate at a larger time scale, requiring long-lasting experiments to reveal them. We conclude that the response of these species to inflicted damage is likely to be linked to their longevity and developmental constraints.

Keywords: *bud removal*, *Centaurea jacea*, *compensatory effects*, *costs of reproduction*, *garden experiment*, *herbivory*, *Hypochaeris radicata*, *meristem allocation*, *phenology*, *resource allocation*, *Succisa pratensis*, *switching*

Introduction

Life history theory predicts that organisms optimise their life time fitness according to the trade-off between current sexual reproduction and growth, survival and future reproduction (Stearns 1989; Bazzaz & Grace 1997). In rosette-producing perennials this means that within each growing season the resources acquired are partitioned between flowering and seed set, production of new rosettes, and growth. Resource allocation towards the various life history functions may differ considerably between species (van Andel & Vera 1977; Bazzaz 1996), depending on environmental variation, life span, competitive dominance, mode of vegetative spread and other factors (Harper 1967). Like in animal life history theory, traditional plant resource allocation theory stated that resources can be allocated towards one life history function only, and that trade-offs between these functions can therefore be expected.

However, this view may not accord with the modular character of plant parts or their ability to be self-sustaining. In plant ecology it has since long been recognised that photosynthesizing plant parts do not only act as resource sinks but also as sources of carbon (Benner & Watson 1989; Vuorisalo & Mutikainen 1999). As a consequence trade-offs between functions are not necessarily apparent. Nowadays, also meristem allocation is regarded as an important feature in interpreting plant strategies (Olejniczak 2001), since it has been shown that, especially in nutrient-rich environments, meristems may be more limiting than resources (Geber 1990; Bonser & Aarssen 1996). Commitment of meristems to reproduction reduces the number of meristems available for vegetative growth. Conversely, dedicating meristems towards vegetative growth can increase the number of available meristems later in life (Watson 1984; Olejniczak 2001). Meristem allocation theory assumes that the costs of reproduction are paid in the currency of meristems rather than the amount of spent resources, e.g. Watson (1984) and Geber (1990). Although both processes are evidently important, still few studies have looked at biomass and meristem allocation concomitantly.

Prati and Schmid (2000) suggested that allocation in plants is essentially a two-step process. The first step involves the developmental decision to differentiate a particular meristem into a vegetative or a sexual structure or to remain dormant (Watson 1984; Duffy et al. 1999). The second step comprises the physiological control over resource flows that determine the sizes of structures, which often includes the abortion of fruits or seeds (Stöcklin 1997). In case it is unclear beforehand what the limiting factor is, both biomass and meristem allocation should be considered when investigating the importance of trade-offs between life-history functions.

Manipulating the effort a plant makes to a certain function can reveal the relative importance of life history functions (Stearns 1989; Ehrlén 1999; García & Ehrlén 2002). In bud removal experiments resources that cannot be used for the development of the removed plant parts become available for other purposes. In general, plants have two options of responding when plant parts are removed; they can either try to compensate for the loss by replacing the lost parts, or switch their

resource investments towards other functions. This choice probably depends on developmental constraints, the expected life span, timing of damage, etc. In the various bud removal experiments performed so far, different responses were found for different species.

Compensatory fruit production after main stem clipping was found by Lennartsson et al. (1998) in *Gentianella campestris* and by Lehtilä and Syrjänen (1995) in two *Melampyrum* species. In the latter experiment flower removal also led to compensatory seed production later in the season. Examples of overcompensation are also known as a result of herbivory (Watson 1995). The release of apical dominance is assumed to be one of the mechanisms behind overcompensation. When the main apex has been removed, the apices behind it will become activated and start to develop and grow (Svensson & Callaghan 1988) and the overall effect can even be an increase in flowering.

Switches in resources allocation after manipulation have been found by Westley (1993) in the tuber-forming species *Helianthus tuberosus*, by Prati and Schmid (2000) in the clonal *Ranunculus reptans*, and in the cantaloupe melon *Cucumis melo* by El-Keblawy and Lovett Doust (1996). A natural trade-off between life

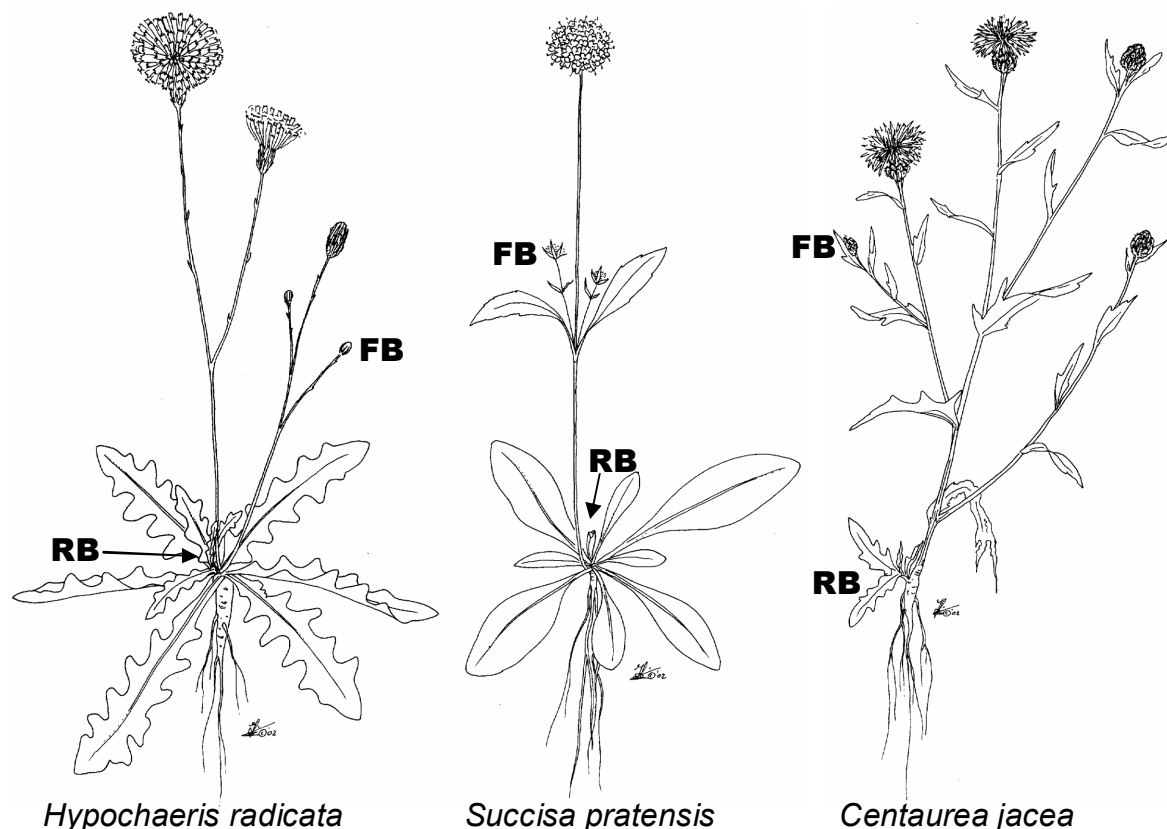


Figure 1.

Drawings of *Hypochaeris radicata*, *Succisa pratensis* and *Centaurea jacea*. The flower buds and rosette buds that were removed in the experiment are indicated with FB and RB respectively. Juul Limpens made the drawings.

history functions in *Tipularia discolor* has been studied by Snow and Whigham (1989). They conclude that flowering and fruiting depleted stored resources that otherwise would have been allocated to growth, vegetative propagation or flowering in the following year. In some cases however, it is hard to make such a distinction, for instance if extra growth also implies more flower bud production (Avila-Sakar et al. 2001). There are several constraints to a plastic response of a plant to inflicted damage, such as meristem preformation (Geber et al. 1997), growth rate, timing of damage (Lennartsson et al. 1998) and plant architecture (Lehtilä & Syrjänen 1995). However, it remains unclear which of these underlying factors determine the choice between compensation and switching and the expression of trade-offs in herbaceous plant species.

In an attempt to increase our understanding of this topic, we performed a bud removal experiment with three perennial grassland herbs (*Hypochaeris radicata*, *Succisa pratensis* and *Centaurea jacea*). These three species all reproduce sexually as well as vegetatively by means of developing new rosettes, which eventually may become independent from the mother rosette. However, they differ in longevity (*H. radicata* is short-lived, whereas the other two are long-lived) and flowering type (*H. radicata* and *C. jacea* flower apically, *S. pratensis* laterally). We addressed the following questions: To what extent do these species compensate for lost plant parts and to what extent do they switch to other functions in response to flower bud removal and side rosette bud removal, respectively? Are changes in biomass and meristem allocation consistent with a trade-off between life history functions?

Materials and methods

Species

We performed a garden experiment with three species: *Centaurea jacea* L. (Asteraceae), *Hypochaeris radicata* L. (Asteraceae) and *Succisa pratensis* Moench (Dipsacaceae) (Fig. 1). They are found in comparable habitats, rather nutrient-poor grasslands (Soons & Heil 2002), and have similar life history choices (clonal growth as well as sexual reproduction), but they differ in life span and flowering type.

Hypochaeris radicata is a relatively short-lived species (de Kroon et al. 1987; Fone 1989); its life span usually does not exceed two seasons. *H. radicata* flowering stalks and new rosettes are formed in the centre of the main rosette; the number of flowering stalks varies considerably (de Kroon et al. 1987). Flowering lasts from spring until late autumn (Fone 1989).

Centaurea jacea is a relatively long-lived perennial, although it has monocarpic shoots. Single individuals have been recorded to survive until at least the age of ten years (Tamm 1956). *Centaurea jacea* rosettes have a single apical flowering stalk. During and after flowering, vegetative side-rosettes form on the rootstock and appear

at the soil surface alongside the main stem (personal observation). Flowering lasts from June until the first frost (van der Meijden 1996).

Succisa pratensis, like *C. jacea*, can survive for many years (Adams 1955; Hooftman & Diemer 2002). *S. pratensis* flowers laterally and usually produces more than one flowering stalk. New rosettes also emerge laterally. This species, which is rather rare in the Netherlands, flowers from late July until October (Vergeer et al. 2003).

Seeds were collected in a *Cirsio-Molinietum* vegetation in the nature reserve 'Bennekomse Meent' near Wageningen (52°01'N, 5°36'E). This remnant of a formerly extensive communal hayfield is mown once a year in the beginning of August, it is not grazed by large herbivores. For a more detailed site description see van der Hoek and Braakhekke (1998). Plants of *S. pratensis* and *C. jacea* were grown from seed in a greenhouse one year before the start of the experiment. Side rosettes were cut from these one-year-old plants and transplanted into the experimental garden in May 2000. *H. radicata* plants were grown from seed, germinating in March 2000. Plants were put

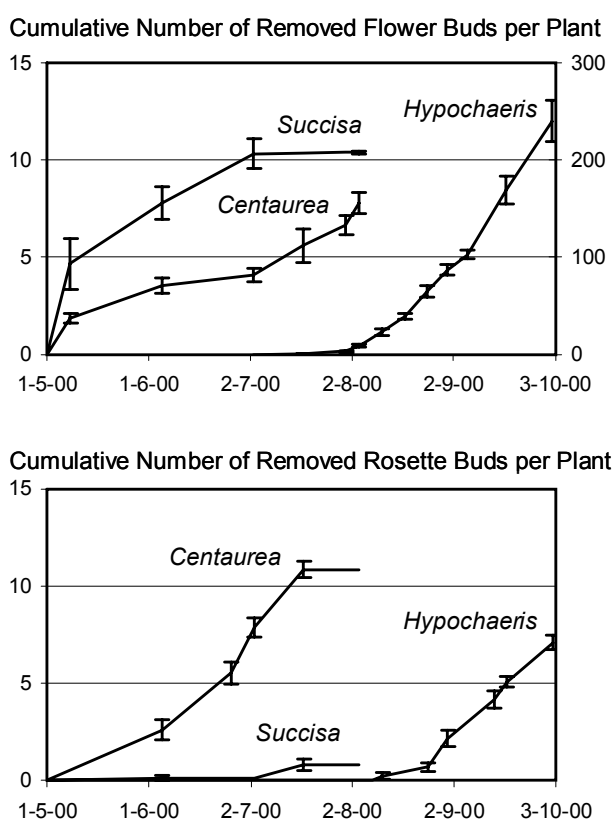


Figure 2.

Cumulative number of removed flower buds and rosette buds per plant of *Centaurea jacea*, *Hypochaeris radicata* and *Succisa pratensis*. Note that the *H. radicata* flower buds are on the right-hand axis. Bars represent \pm standard error of the number of removed buds per plant on that particular day. Small rosette and flower buds found at harvest are not depicted in this figure, but are included in Fig.3.

at an interval of approximately 0.5 m in nutrient poor soil in a hexagon of *Molinia caerulea* plantlets (5 shoots each) in order to mimic natural conditions.

Experimental design

The experiment was performed during the growing season of 2000. We used 15 plants per treatment and species. To minimise the effect of genetic variation and initial size, three new-born rosettes with approximately the same weight, descending from a single parent, and number of leaves and stems, were selected to form a 'trio'. Plants from each trio were randomly distributed over the three treatments. Trios of different size were used, as to be able to study the effect of initial size. For *H. radicata*, the 'trios' consisted of seedlings that descended from one flower head. A randomised block design was used.

The plants were subjected to one of three treatments. The first was a flower bud removal treatment. We removed only the buds of flower heads (hereafter called flower bud), since removing the whole flowering stalk would probably affect a plant's photosynthetic capacity (Fig. 1). Flower buds were removed when they were large enough to be removed without damaging the rest of the flowering stalk. Flower bud removal was applied approximately three times each month until the end of the experiment. The removed flower buds were counted per plant, dried at 70°C for two days and weighed. The second treatment was a rosette bud removal. For *H. radicata* and *S. pratensis*, that produce new rosette buds above the soil surface, the buds were removed apically and laterally from the main rosette, respectively. For *C. jacea*, that forms side-rosettes on the rootstock just beneath the surface, rosette buds were cut off after gently removing the soil around the rootstock. Rosette buds were counted, dried and weighed like the flower buds. The third treatment was a control, in which neither flower buds nor rosette buds were removed.

The number of flower buds, rosette buds, flowers and rosettes was recorded for each individual. For the removal treatments this was done partly during the experiment by counting and weighing the removed parts. Harvest took place just after most seeds had ripened; harvest date differed between the species (8th of August for *C. jacea* and *S. pratensis* and October 3rd for *H. radicata*), since *H. radicata* has a more prolonged flowering season. At harvest plants were dug out, and above and below ground parts were weighed. To be able to determine the number and weight of the full-grown flower heads with flowers and seeds that otherwise would have fallen from the plant during the experiment, small plastic wire-netting bags were placed over the flower heads.

Data analysis

We used an analysis of variance model with species, treatment and trio (plants of identical genotype and same initial size) as factors for the dry weights. Number of flower buds and of rosette buds were analysed with a non-parametric Wilcoxon Signed

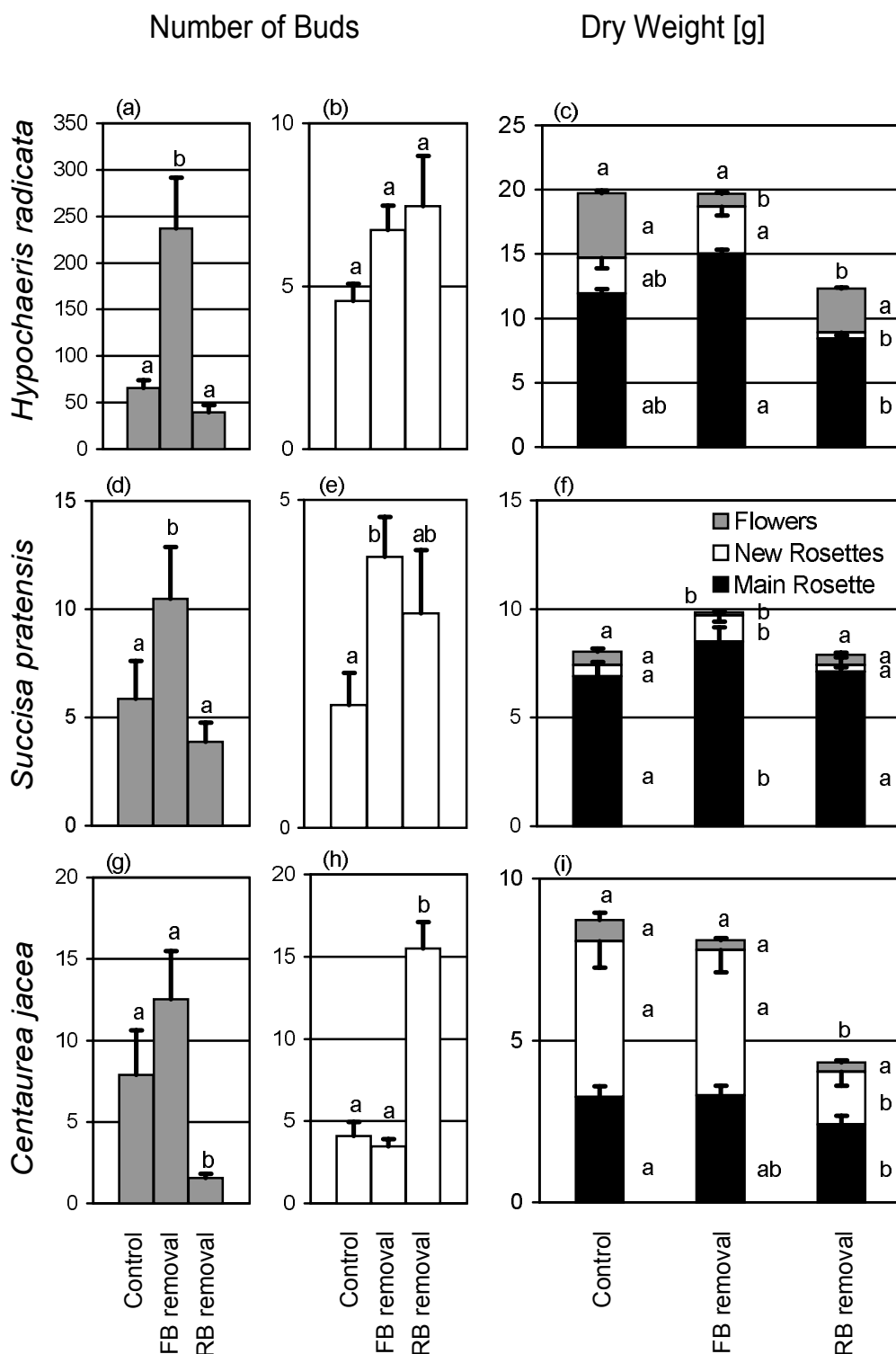


Figure 3.

Number and dry weight of rosettes and flowers per harvested plant, divided into main rosette (black), new rosettes (white) and flowers (dashed). The treatments (with $n=15$ plants) were control (Control), flower bud removal (FB removal) and rosette removal (RB removal). Numbers of buds were analysed with a Wilcoxon Signed Ranks Test with Bonferroni correction (Fig. 3 a, b, d, e, g and h, see also Table 2). ANOVAs on the dry weight at harvest were performed with Treatment and Trio (plants of identical genotype or seed family and same initial size) as explaining factors (Fig. 3 c, f and i, see also Table 1). Note that the weight of the flowers for FB removal and the weight of the rosettes for RB removal should be interpreted as the weight of the removed plant parts. Within each graph a different letter with the same plant part denotes a significant treatment effect on that plant part.

Ranks Test, since the data were not normal or Poisson distributed, and their variances were significantly not homogeneous. This test was used to make pair-wise comparisons between the plants in one trio. In order to compare all three treatments, the Bonferroni correction was applied: the significance level is lowered by division by the number of compared tests ($\alpha = 0.05 / 3 = 0.017$). There was no significant block effect. Four *H. radicata* plants died in the course of the experiment and were left out of the analyses.

Results

Timing and extent of flowering and rosette bud formation

Most plants flowered and produced new vegetative rosettes. Not all plants (but at least 2/3 in every Species-Treatment combination) flowered or formed new rosettes and therefore some plants could not receive the removal treatment that they were assigned to. The results are thus a conservative estimate of the real effects of the treatments.

The emergence of new rosettes occurred after the onset of flowering in all three species (Fig. 2). *Hypochaeris radicata* produced several new ones for every removed flower bud. New rosettes were formed in August and September. *H. radicata* continued flowering and producing side rosettes until the plants were harvested. The *Succisa pratensis* plants produced flowers (about three flower heads per plant) in May and June, whereas the production of rosettes started in July. The *Centaurea jacea* plants produced flower buds on their main stem in May and the beginning of June, but there was a second period of flowering in July, when the side rosettes started to flower.

Effect of bud removal on dry weights

The three species responded markedly different to flower bud removal (Table 1, Fig. 3). In *H. radicata* we see a strong trend ($p = 0.065$) of an increase in total vegetative weight when flower buds were removed (Fig. 3c), but in *C. jacea* none of the biomass responses were significant (Fig. 3i). Only in *S. pratensis* flower bud removal led to a concomitant increase in total plant weight. The weights of the main and new rosettes increased (Fig. 3f). Since trio was an important explaining factor for dry weight in *S. pratensis* (Table 1), we tested for bivariate correlation between initial fresh weight and dry weight at harvest ($n = 45$), resulting in a Pearson's correlation coefficient of 0.437 with a two-tailed significance of 0.003.

Rosette bud removal decreased total dry weight in *H. radicata* and *C. jacea* (Fig. 3c,i). Flower weight was not affected by the smaller biomass investment in side rosettes in these species. Main rosette weight was slightly lower for *H. radicata* and

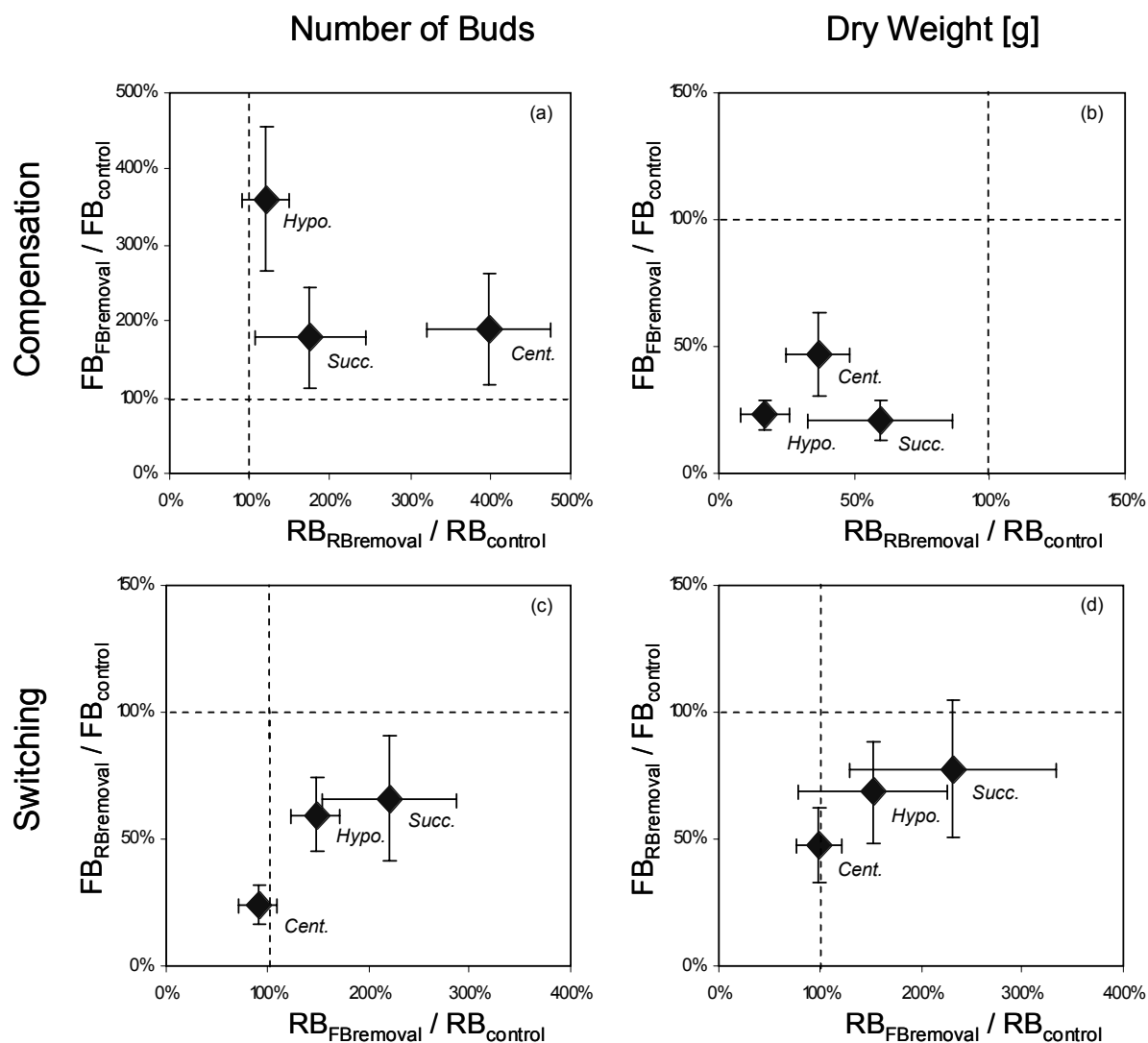


Figure 4.

Summary of the effects of continuous removal of either flower or rosette buds on the number and dry weight of flowers and new rosettes of (*Hypo.*) *Hypochaeris radicata*, (*Succ.*) *Succisa pratensis* and (*Cent.*) *Centaurea jacea*. In each diagram a treatment effect on flower allocation is plotted on the y-axis, and a treatment effect on new rosette allocation on the x-axis. Compensation for continuously removed buds is calculated as the percentage change in the number (a) and dry weight (b) of that bud type, compared to the control group. Trade-offs are calculated as the percentage change in the number (c) and dry weight (d) of a certain bud type, when the other type of buds is removed. The 100%-lines indicate no effect of the treatments. Dots and bars represent species means and standard errors on basis of within trio comparisons.

$FB_{Control}$ = flower (buds) of the control group
 $FB_{FBremoval}$ = flower (buds) of the flower bud removal group
 $FB_{RBremoval}$ = flower (buds) of the rosette bud removal group
 $RB_{Control}$ = new rosettes of the control group
 $RB_{FBremoval}$ = new rosettes of the flower bud removal group
 $RB_{RBremoval}$ = new rosettes of the rosette bud removal group

Table 1.

ANOVAs on dry weight at harvest for all species together and for each species separate (*Hypochaeris radicata*, *Succisa pratensis* and *Centaurea jacea*). Species, Trio (plants of identical genotype and same initial size) within Species, Treatment (control, flower bud removal, and rosette bud removal) and Species x Treatment were used as explaining factors of the variation in dry weight (total, main rosette, new rosettes, and flower heads and buds).

Source of Variation	Total plant			Main Rosette			New Rosettes			Flower Heads&Buds		
	df	MS	F	df	MS	F	df	MS	F	df	MS	F
<i>All plants</i>												
Species (S)	2	1125.1	68.4 ***	2	728.1	87.5 ***	2	81.9	20.6 ***	2	103.7	93.3 ***
Trio (within S)	42	37.2	2.3 ***	42	18.1	2.2 **	42	5.7	1.4 (*)	42	2.4	2.2 ***
Treatment	2	266.2	16.2 ***	2	107.2	12.9 ***	2	66.0	16.6 ***	2	28.4	25.6 ***
S x Treatment	4	62.6	3.8 **	4	43.9	5.3 ***	4	9.2	2.3 (*)	4	16.3	14.6 ***
Error	74	16.5		77	8.3		77	4.0		79	1.1	
<i>H. radicata</i>												
Treatment	2	271.2	6.8 **	2	170.5	7.4 **	2	38.3	5.2 *	2	57.1	16.7 ***
Trio	14	76.8	1.9 (*)	14	36.1	1.6	14	8.8	1.2	14	6.7	2.0 (*)
Error	24	40.1		24	23.1		24	7.4		24	3.4	
<i>S. pratensis</i>												
Treatment	2	16.6	4.7 *	2	11.5	5.4 *	2	3.3	6.3 **	2	0.9	11.0 ***
Trio	14	21.5	6.1 ***	14	16.2	7.6 ***	14	0.9	1.6	14	0.4	4.6 ***
Error	28	3.6		28	2.1		28	0.5		28	0.1	
<i>C. jacea</i>												
Treatment	2	76.9	10.9 **	2	5.6	5.4 *	2	39.2	8.6 **	2	0.4	3.2 (*)
Trio	14	13.4	1.9 (*)	14	2.0	1.9 (*)	14	7.5	1.6	14	0.2	1.6
Error	22	7.1		25	1.0		25	4.6		27	0.1	

significantly lower for *C. jacea*, under rosette bud removal, but this treatment did not affect the weight of any part of the *S. pratensis* plants.

Effect of bud removal on numbers of flowers and rosettes

Flower bud removal caused an increase in the number of flower buds in all three species, although this was not significant for *C. jacea* (Fig. 3a, d, g). The most spectacular increase was seen in *H. radicata* that produced on average four times as many flower buds when flower buds were continuously removed. *H. radicata* plants in the flower bud removal group also produced more flowering stems (17.2 vs. 10.7 for the control group, $p = 0.059$) and showed more branching of stems. The number of side rosettes in *S. pratensis* increased when flower buds were removed.

Rosette bud removal induced an increase in the number of rosette buds (Fig. 3b, e, h). Especially in *C. jacea*, which produced four times more rosette buds if rosette buds were removed. Flower production declined for all three species when rosette buds were removed, and most clearly so in *C. jacea*, probably because for these plants new rosettes could not contribute to flowering (Fig. 3g). For *H. radicata* compensation for lost rosette buds was not significant.

Plotting the effects of both treatments in the same graph (Fig. 4) can highlight the hypothesised effects of flower or rosette bud removal, compensation or switching. *H. radicata* shows the strongest compensation for lost flower buds, but the weakest for rosette buds, whereas *C. jacea* shows a strong compensation for rosette buds and the weakest for flower buds (Fig 4a). *Succisa pratensis* replaces flower buds and rosette buds at the same degree, it produces twice as many buds as it would have if buds had been allowed to develop (Fig 4a). However, this compensatory effect was not seen in the dry weights (Fig 4b), since flower buds were removed at a very small size.

A switch from rosette bud production towards flowering, in other words an increase in number of flowers or total flower weight when rosette buds were removed, did not occur in our experiment. All species and especially *C. jacea* showed a decrease in flower number and weight when rosette buds were removed (Fig. 4c and 4d). Switching from flowering to rosette production did occur in *H. radicata* and *S. pratensis*, but not in *C. jacea*.

Discussion

Our study revealed that inhibition of flowering or development of new rosettes had very different impacts on the three perennial herbs *Hypochaeris radicata*, *Succisa pratensis* and *Centaurea jacea*. The significantly different responses of the three species with respect to meristem decisions and biomass allocation reflect differences between the species in life history traits and in constraints on allocation.

Compensation

All three species responded to flower bud removal by compensating for lost flower buds, but not all to the same extent. *Hypochaeris radicata* showed a 3-4 fold increase in the number of flower buds formed. This, together with the fact that this species had the largest proportional reproductive effort in terms of biomass allocation to flower heads in the control treatment, indicates the great importance of seed production for this species. This may be related to its short life span, since the urge for sexual reproduction in a certain growing season is assumed to be larger for short-lived species (Ehrlén & van Groenendael 1998). De Kroon et al. (1987) showed that the growth of *H. radicata* populations is highly depending on the sexual pathway, unlike many other perennials.

Our results are consistent with compensatory effects shown in other experiments on short-lived species, such as the hemiparasitic annuals *Melampyrum pratense* and *M. sylvaticum* (Lehtilä & Syrjänen 1995), the biennial *Gentianella campestris* (Lennartsson et al. 1998) and the annual *Cucurbita pepo* ssp. *texana* (Avila-Sakar et al. 2001). Rosette bud removal led to a minor increase in number of

Table 2.

Wilcoxon Signed Rank Tests between Treatments (Control, Flower Bud removal, and Rosette Bud removal). Number of flower or rosette buds produced during the experiment were compared per Trio (plants of identical genotype and same initial size). Note that to determine significant differences between the three Treatments (as in Fig. 3) the Bonferroni correction is applied: the significance level is lowered by division by the number of compared tests ($\alpha = 0.05 / 3 = 0.017$).

Number of	Comparison	<i>H. radicata</i>		<i>S. pratensis</i>		<i>C. jacea</i>	
		Z	p	Z	p	Z	p
Flower Heads&Buds	Control - FB removal	-2.900	0.004	-2.805	0.005	-1.848	0.065
	Control - RB removal	-2.001	0.045	-1.889	0.059	-2.397	0.017
	FB removal - RB removal	-3.180	0.001	-2.674	0.007	-3.415	0.001
Side Rosettes	Control - FB removal	-1.843	0.065	-2.559	0.011	-0.387	0.698
	Control - RB removal	-1.636	0.102	-1.299	0.194	-3.413	0.001
	FB removal - RB removal	-0.035	0.972	-0.916	0.360	-3.410	0.001

rosette buds in *H. radicata*, probably because only few rosettes were removed. We conclude from the high branching rate, the flexible number of flowering stems and the strong and fast flower bud compensation that *H. radicata* is not noticeably limited by meristem availability. This, as well as the high growth rate, enables this perennial to respond quickly to damage to flower buds, for instance inflicted by herbivores. In the case of a yearly mown nature reserve like the place of origin of our plants, this adaptive response may also be beneficial, because the plant can start flowering again soon after having been mown. At the same time this short-lived species tends to increase its vegetative weight and the number of new rosettes, which has been shown to contribute critically to plant fitness through enhanced seed production (de Kroon et al. 1987).

For *C. jacea*, compensation is shown, but only with respect to the new rosettes. This monocarpic species depends on new rosettes for survival since the main rosette dies after flowering. For *C. jacea* new rosettes may be more important than flowering, since due to the long life span of the genet it can wait until next year when circumstances may be more favourable.

Succisa pratensis shows some compensation, but to a lesser extent than the other two species. When buds are removed, it only produces twice as many buds compared to a control situation. This might be linked to a more strict developmental programme, indicated by the symmetric growth form as well as the strong correlation between initial size and dry weight at harvest that was shown by the results of the correlation test. However, since within each trio both initial size and genotype are more or less identical, we cannot conclude whether variation between trios is due to initial size or to genotypic effects.

Since the main rosette is polycarp, there is no necessity to immediately compensate for lost rosettes; the plant does not depend on new rosettes for survival like *C. jacea*. Unlike *H. radicata*, the long-lived rosette of *S. pratensis* can postpone sexual reproduction, what might explain the lower tendency to compensate for lost flowers.

Switching

Bud removal also caused shifts in allocation towards other life history functions in *H. radicata* and *S. pratensis*. In *H. radicata*, we see a tendency to shift from sexual reproduction towards vegetative growth and new rosettes when flower development is prevented. The number of new rosettes increased as a result of flower bud removal and the total weight of new rosettes also tended to increase, but there is no effect at total plant weight. A similar switch in allocation was demonstrated in an inflorescence bud removal experiment on the short-lived tuber forming species *Helianthus tuberosus* (Westley 1993). However, de Kroon et al. (1987) found that new rosettes are very important for current reproduction in *H. radicata*. New rosettes can therefore be seen as a way of switching and as a way of compensating indirectly for lost flowers. This means the plants switched to an increased resource allocation into the new rosettes, and at the same time compensated for lost flower buds.

In *S. pratensis*, flower bud removal caused an increase in the number of side rosettes, even stronger than the switch in *H. radicata*. For this longer-lived species this investment will probably pay off in later years, whereas for the short-lived *H. radicata* the relative benefit of compensation for lost flower buds is more immediate. Flower bud removal also increased the weight of the whole plant as well as the weights of the separate parts in *S. pratensis*. Prati and Schmid (2000) found a similar increase in plant size as a result of flower bud removal for the clonal herb *Ranunculus reptans*, while proportional allocation towards the several functions did not change. Switches in biomass allocation towards vegetative size are an expression of costs of sexual reproduction, and have been described by Ehrlén and van Groenendael (2001) and El-Keblawy and Lovett Doust (1996) amongst others.

For *C. jacea*, flower bud removal did not cause a switch towards production of new rosettes. An explanation could be that only a small part of the plant's resources is used for the development of a small flower bud to a flower head with seeds, and thus only a relatively small amount of extra resources becomes available when flowering is prohibited. As is shown by the large response in the rosette removal treatment, meristem preformation cannot explain the lack of response. The decrease in main rosette weight in response to rosette bud removal in *C. jacea* may be explained by assuming that the lower part of the main stem serves as a storage organ, which is depleted by the formation of compensatory rosette buds.

Timing has been recognised as an important factor determining the response of a plant to damage (Lehtilä & Syrjänen 1995). Response to damage may even be restricted to a certain period in the season (Lennartsson et al. 1998). In *S. pratensis*, most of the rosette formation took place after the flowering peak. Thus, it is hardly surprising that the impact of removal of new rosettes on flowering was very small, since decisions concerning allocation to flowering and meristem development were made before side rosettes were removed. This timing of allocation and of meristem activity might be a major constraint on the expression of trade-offs between allocation to either function.

In conclusion, we argue that both meristem and biomass allocation play an

important role in determining the response to bud removal, in terms of the choice between compensating and switching. Our results suggest that the degree of compensation and the expression of life history trade-offs are related to longevity and developmental constraints.

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Size-dependent and -independent plant reproductive allocation in response to successional replacement

Eelke Jongejans, Hans de Kroon & Frank Berendse

Summary

Perennial herbs have been hypothesized to have two alternative options when they face the risk of being outcompeted in the course of succession: escape to other sites through seed dispersal, or persist by investing relatively more in vegetative growth. The hypotheses implicitly assume that costs of sexual reproduction prevent plants from escaping and persisting simultaneously. We test these hypotheses in a three-year garden experiment with four perennials (*Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*) by growing them in the midst of a tall tussock-forming grass (*Molinia caerulea*) that may successional replacement in their natural habitat. Costs of sexual reproduction were significant since continuous bud removal enhanced total biomass or rosette number in all four species, while storage biomass increased slightly in the two most long-lived species. To mimic succession we added nutrients, which resulted in tripled grass biomass and higher death rates in the shorter-lived species. Three species increased their sexual reproductive allocation irrespective of size, thus supporting the escape strategy, but not at the expense of allocation to storage, which increased even in *C. jacea*. The strong size-dependency of both sexual reproduction and storage suggests that the two strategies collapse into a single syndrome: in response to succession plants have to increase in size for both survival and increased seed production.

Keywords: *Centaurea jacea*; *Cirsium dissectum*; *Costs of sexual reproduction*; *escape strategy*; *Hypochaeris radicata*; *persistence strategy*; *sexual reproductive allocation*; *size dependency*; *succession*; *Succisa pratensis*

Introduction

Succession is the gradual replacement of one plant community by another, and is characterized by the accumulation of plant biomass and soil nutrients (Crawley 1997).

On the time scale of succession plant populations are ephemeral, and will eventually go locally extinct (Tilman 1987; Falinska 1991), as early successional plant species are gradually outcompeted by taller competitors that accumulate biomass (Berendse et al. 1992; Roem & Berendse 2000). It is unclear however whether and how plant individuals respond to the risk of successional replacement. Perennial plants that are gradually suppressed have long been hypothesized to have two alternatives (Ogden 1974; Abrahamson 1980): 1) to form dense and non-invadable populations and persist, or 2) to escape to new areas. To our knowledge this long-standing hypothesis has never been explicitly tested. Here we rephrase this hypothesis into general predictions within the framework of allometric allocation, and present the results of a three-year garden experiment with four herbaceous perennials, intended to test these predictions.

The rationale behind these alternative predictions was deduced from the observation that during secondary succession, plant traits of the current species change on average. With increasing successional stage vegetative growth and survival become more and more important, at the expense of sexual reproduction and seedling recruitment (Harper 1977; Abrahamson 1979; 1980). These authors argued that plant species that alter their growth pattern could survive to the next successional stage. Alternatively plant species can escape in space via seed dispersal to areas that are still in an early successional stage (Ogden 1974). The first alternative is realized by increasing the likelihood of persistence through increased plant size, increased storage and clonal growth at the expense of short-term sexual reproductive allocation but insuring life-time reproductive effort. And the latter alternative is realized by increasing sexual reproduction and seed dispersal. The predicted shifts in allocation are relative in nature and are not all or nothing decisions.

Some circumstantial evidence does exist for these hypotheses: the success of many clonal perennials may be interpreted to be a consequence of the persistence strategy (Prach & Pyšek 1999). Species as *Phragmites communis* and *Pteridium aquilinum* are extreme examples of clonal species that can form persistent populations, thereby preventing the shift to new successional stages for extended time periods (Marrs et al. 2000). Analyzing within-species variation Falinska (1991) showed that several wet meadow species have a decreased percentage of flowering individuals in consecutive successional phases, from meadows to willow bushes. Studies on the monocarpic perennial *Dipsacus sylvestris*, by contrast, support the escape hypothesis as they find higher seed production but lower seedling establishment probabilities and decreased individual growth rates in populations with high competition by herbaceous dicots and shrubs, relative to populations characterized by low competition (Werner & Caswell 1977; de Kroon et al. 1986). However, the hypothesized phenotypic responses at the individual level have rarely been tested experimentally (but see Holler and Abrahamson (1977) for an example of decreased vegetative reproductive effort under manipulated high plant densities).

Underlying Abrahamson's (1980) hypotheses is a trade-off between seed production and vegetative reproduction. Costs of sexual reproduction, i.e. any

reduction in fitness parameters like survival, growth, size or future reproduction due to resource allocation to sexual reproduction, are crucial for these alternative hypotheses because in the absence of costs plants could change both modes of reproduction independently. Recent studies have shown that trade-offs between different life history functions can be masked by the allometric size-dependent relationship between plant size and investment into e.g. sexual reproduction (van Noordwijk & de Jong 1986; Ågren & Willson 1994; Reznick et al. 2000; Ehrlén & van Groenendael 2001; Worley et al. 2003). In many species a threshold size needs to be reached, after which sexual reproductive biomass often linearly increases with vegetative plant size (Weiner 1988; Hartnett 1990; Méndez & Obeso 1993; but see Klinkhamer et al. 1992 for a non-linear increase). But when plant size is accounted for, trade-offs between life history functions can be found, as is evidenced by manipulative experiments that show switches from one function to another life history function (Westley 1993; Primack & Stacy 1998; Obeso 2002; Hartemink et al. 2004) or by studies under natural,

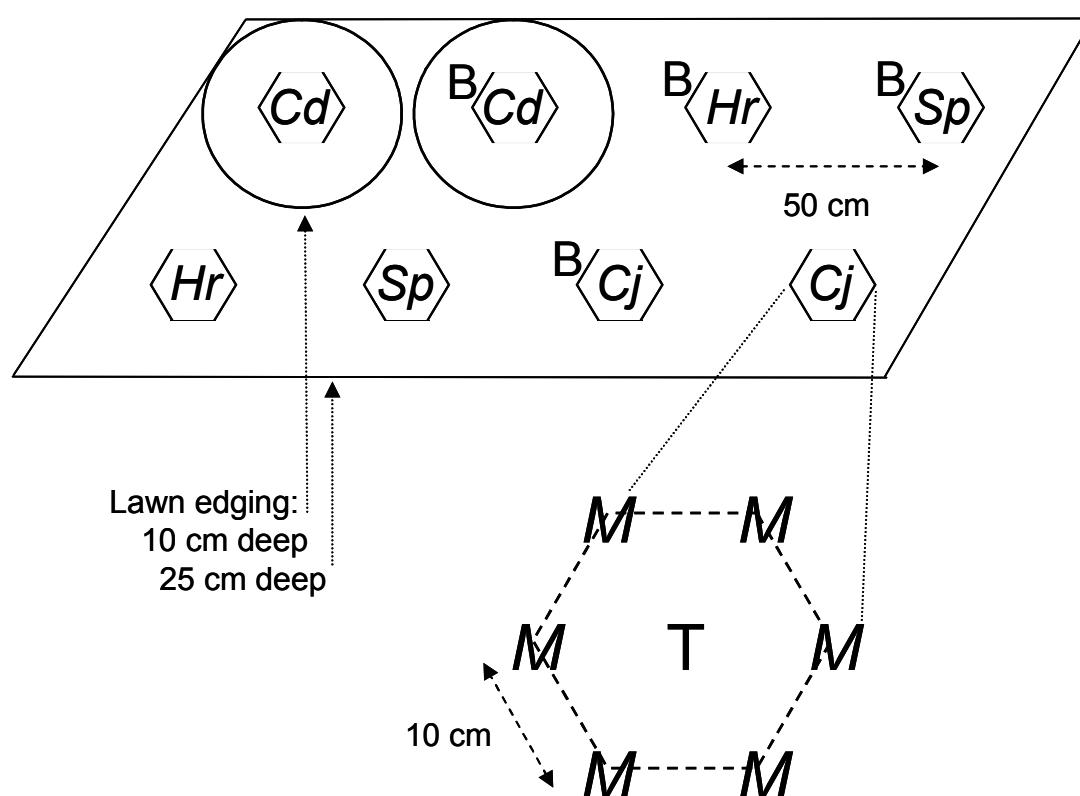


Figure 1.

Schematic design of part of the experiment. The subblock of eight plants represented here either received extra nutrients, or served as control. Each of the twenty blocks contained two adjacent subblocks with contrasting nutrient treatment. Within a subblock the eight species by bud removal combinations were randomly distributed over the positions. The subblocks were fenced with 25 cm deep lawn edging to prevent root growth or leakage of nutrients between nutrient treatments. Ten cm deep lawn edging (50 cm diameter) kept the rhizomatous *Cirsium dissectum* from growing into other plots. Around each target plant six cuttings of *Molinia caerulea* were planted in a hexagon at 10 cm intervals. Hr = *Hypochaeris radicata*; Cd = *Cirsium dissectum*; Sp = *Succisa pratensis*; Cj = *Centaurea jacea*; T = target plant; M = *M. caerulea*; B = flower bud removal.

undisturbed conditions (Snow & Whigham 1989; Willems & Dorland 2000; Rydgren & Økland 2002). However, costs of sexual reproduction may still not become apparent when these costs decrease with increasing nutrient availability (Reekie 1991) or when sexual reproductive structures are self-supporting or even lead to enhanced vegetative growth (Reekie & Bazzaz 1987; Watson et al. 1997).

For the four perennial plant species studied, our first aim is to test for costs of sexual reproduction in order to verify whether the prerequisites for the hypotheses of Ogden and Abrahamson are met. We use the method of flower bud removal (Obeso 2002) to investigate if plants switch to increased size, storage, and vegetative offspring number when flowering and seed set are inhibited.

The allometric relationships also have consequences for the hypothesized shifts in reproductive allocation patterns. Acknowledging this size-dependency Sugiyama and Bazzaz (1998) described three ways in which reproductive allocation can differ between two groups of plants: a) as the result of a simple allometric relationship between reproductive allocation and plant size, b) by a size-independent shift in reproductive allocation, or c) by an increase in the proportion of the resources allocated to sexual reproduction. Only the latter two scenarios can be regarded as real phenotypic (plastic) responses of the allocation pattern to changing conditions. Consideration of size-dependency leads to the following reformulation of the hypothesis of Ogden (1974) and Abrahamson (1980): *when overall biomass of neighboring plants increases during succession, perennial plants shift their allometric relationships in such a way that they allocate more biomass to seed production (escape strategy) and less to vegetative and storage organs, compared to unaffected plants of equal size.* Alternatively (persistence strategy), perennial plants may shift their allometric relationships in such a way that they allocate more biomass to vegetative and storage organs and less to seed production, compared to unaffected plants of equal size.

Our second aim is to test these hypotheses in a three-year garden experiment with four perennial herbs from nutrient-poor meadows in the Netherlands. These *Cirsio dissecti-Molinietum* grasslands harbor a species-rich, early-successional community (Schaminée et al. 1996) and is maintained in this stage by mowing and hay removal and sometimes additional grazing. Succession starts by biomass accumulation of the dominant grass species. Later also shrubs start to dominate. High levels of nitrogen deposition as they occur in the Netherlands accelerate succession (Tilman 1987; Crawley 1997), and especially subordinate herb species are lost (Roem et al. 2002). In our experiment the target herb plants compete with a dominant, tall grass with which they co-occur. In order to mimic the accumulation of plant biomass and nutrient availability during natural succession with high atmospheric deposition, we fertilized half of the plots. Under fertilized conditions the grass species is expected to outcompete the herbs (Berendse et al. 1992; van der Hoek et al. 2004).

Materials and methods

The hypotheses on costs of sexual reproduction and shifts in allocation pattern were investigated in a three-year (2000-2002) garden experiment with four perennials: *Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*. Allocation of biomass to four parts of the plants was studied: sexual reproductive structures (flower heads, seeds, and buds of flower heads), clonal organs (rhizomes; only in *C. dissectum*), vegetative plant parts (flowering stems, stem leaves, rosette leaves, and roots), and storage organs (the caudex, i.e. the persistent rootstock to which the rosette leaves, stems and roots are attached).

Study system and species

Cat's ear, *Hypochaeris radicata* L. (Asteraceae), is a relatively short-lived perennial (Fone 1989). *Hypochaeris radicata* flowering stalks and new rosettes are formed clonally by branching of the taproot; the number of the leafless flowering stalks varies from one to several (de Kroon et al. 1987). Flowering starts in June and continues until autumn.

Meadow Thistle, *Cirsium dissectum* (L.) Hill (Asteraceae), is a rhizome-forming clonal plant, with monocarpic rosettes. Most daughter rosettes are formed at the end

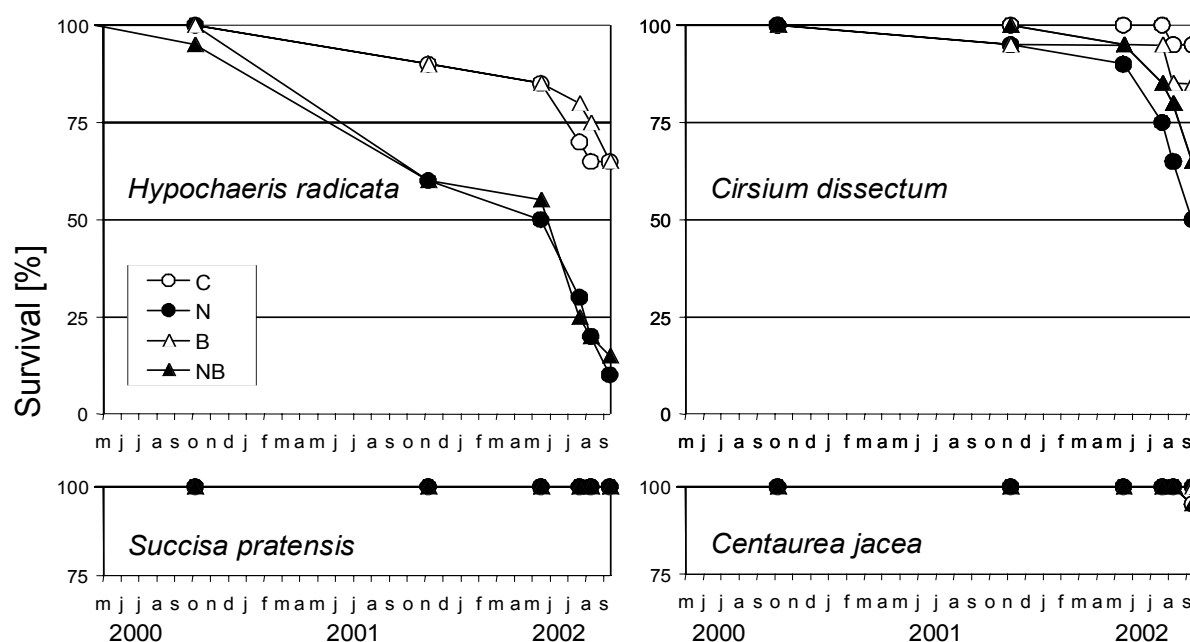


Figure 2.

Survival percentages in time. A letter on the x-axis stands for the 15th day of the month. C=control. B=bud removal; N=nutrients added; N+B=both bud removal as nutrients added.

of long rhizomes, but new rosettes can also be formed at the base of the old rosette. *Cirsium dissectum* normally forms only one, apical flower head in June. In the Netherlands *C. dissectum*, is a rare and endangered species (Red List 2 (van der Meijden 1996)) due to the decline of its habitat (Lucassen et al. 2003).

Devil's-bit Scabious, *Succisa pratensis* Moench (Dipsacaceae), rosettes are polycarpic and can survive for many years (Adams 1955; Safford et al. 2001; Hooftman & Diemer 2002). *Succisa pratensis* laterally forms up to four flowering stalks and flowers from July till September. New rosettes also emerge laterally from the caudex.

Knapweed, *Centaurea jacea* L. s.l. (Asteraceae), is a relatively long-lived perennial, although it has monocarpic shoots. Single individuals have been recorded to survive at least ten years (Tamm 1956). *Centaurea jacea* has a single apical flowering stalk and flowers from June until autumn. During and after flowering, vegetative side-rosettes are formed on the woody rootstock and appear at the soil surface alongside the flowering stem. Subsequently these rosettes can form new stems that grow horizontally for several centimeters before growing vertically. All four target species have composite flower heads. Only the seeds *H. radicata* and *C. dissectum* are plumed and adapted to wind dispersal (Soons & Heil 2002).

Purple Moor-Grass, *Molinia caerulea* (L.) Moench (Poaceae), is a tussock forming, tall grass, which occurs in nutrient-poor grasslands and grass heaths. *Molinia caerulea* starts to dominate when nutrient deposition is high (Berendse & Aerts 1984; Aerts et al. 1990), especially when fields are abandoned.

Plant material

Seeds of the four target species were collected in 1998 in the nature reserve 'Bennekomse Meent', a nutrient-poor grassland near Wageningen in the Netherlands (52°01'N, 5°36'E; van der Hoek & Braakhekke 1998). Cuttings of *M. caerulea* were collected at the same locality. Plants of *C. jacea*, *S. pratensis* and *C. dissectum* were grown from seed in a greenhouse one year before the start of the experiment. In May 2000 newly formed rosettes of these plants were carefully broken off. For *H. radicata* two-months-old seedlings were used. In order to allow for direct comparisons between the bud removal treatment (see below) and the undisturbed plants, all cuttings and seedlings were grouped in pairs of similar initial size, and of the same genetic identity, or, in case of *H. radicata*, grown from seeds of the same mother plant. One plant of each pair was assigned to the bud removal treatment, the other to the untreated group. Each pair of plants was either assigned to the nutrient addition treatment or to the low nutrients treatment. The size-dependency of allocation responses (Fig. 2 of Chapter 1) was taken into account by starting the experiment with a range of plant sizes rather than selecting for equally-sized plants. The cuttings and seedlings were transplanted into an experimental garden of Wageningen University.

The 320 plants were randomly placed in a randomized block design: four species * two bud removal treatments * two resource treatments * 20 replicates (Fig.

1). The interval between the target plants was 50 cm. Around each target plant six *M. caerulea* clumps of four shoots each were placed in a hexagon with sides of 10 cm. Lawn edging was placed 10 cm deep in a 50 cm-diameter circle around the *C. dissectum* plants to prevent this rhizomatous species from growing through the whole garden. Measurements on 20 additional plots indicated that these circles of lawn edging did not affect the biomass increments of *M. caerulea* (results not shown).

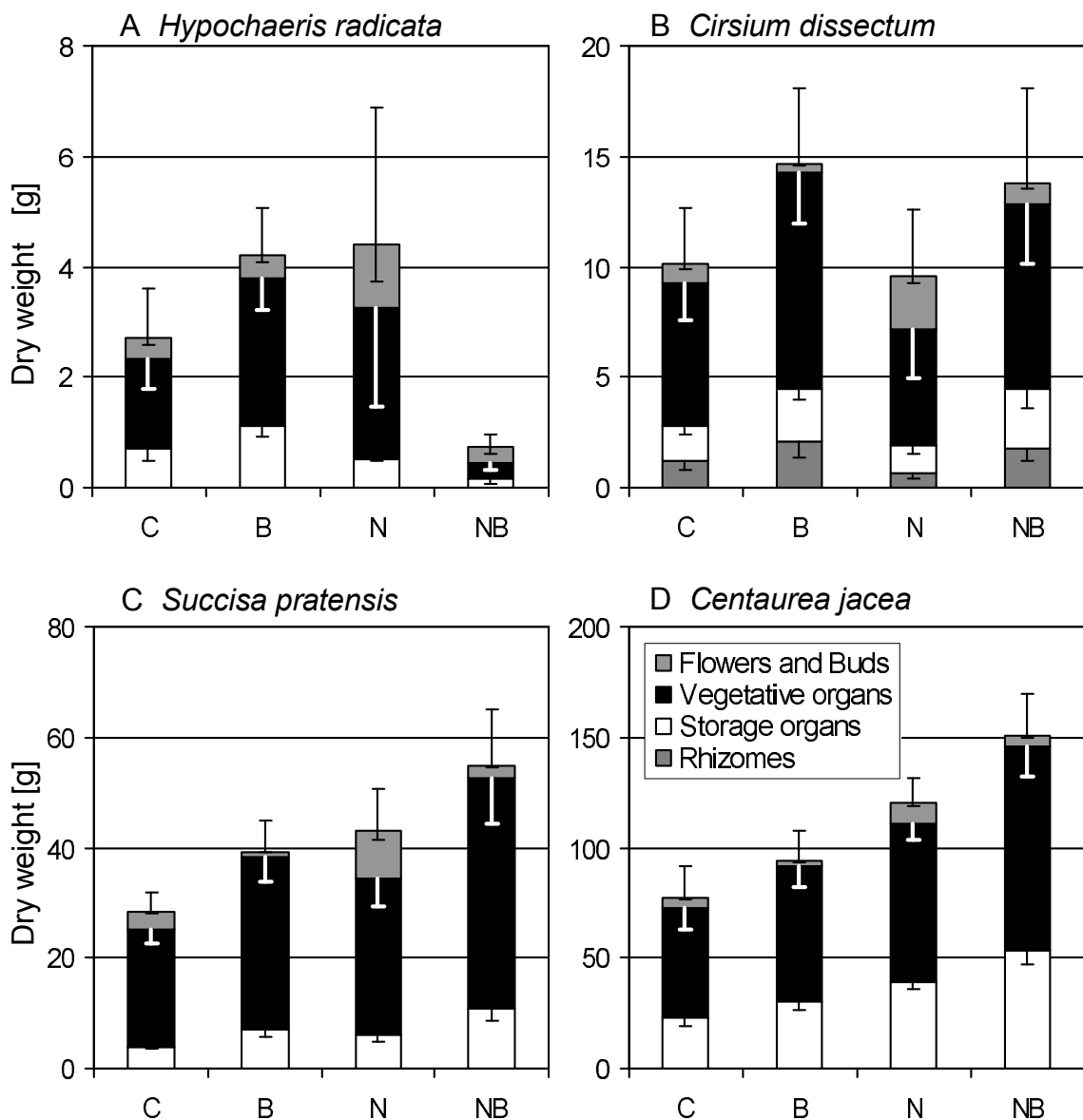


Figure 3.

Dry weight [g] at harvest divided into sexual reproductive tissue (flower buds, flower heads and seeds), vegetative plant parts (leaves, stems and roots), storage organs (caudex), and rhizomes (*C. dissectum* only). Downward error bars denote standard errors of the mean weight of plant parts; the upward error bar denotes the standard error of the mean total weight. C=control. B=bud removal; N=nutrients added; NB=both bud removal as nutrients added. Please note that the N and NB groups of *H. radicata* consist of two and three plants only.

Treatments

Nutrient enrichment was applied to half of the plants in the second and third year, allowing the plants to establish under the same conditions in the first year of the experiment. Nutrient solution was applied to a circular area of 50 cm diameter around the target plants. The Hoagland's stock solution contained KNO_3 , $\text{Ca}(\text{NO}_3)_2$, MgSO_4 , and $\text{NH}_4\text{H}_2\text{PO}_4$ (Gamborg & Wetter 1975). The solution was applied in three portions within two months at the beginning of the growing season and was equivalent to $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, which is about three times the atmospheric deposition in Dutch agricultural landscapes (Bobbink et al. 1998; van Oene et al. 1999). Van der Hoek et al. (2004) found significant shifts in the vegetation composition in the field when they applied $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. In our experiment all plants that were assigned to the high nutrient treatment were placed together in each of the 20 blocks and surrounded with 25 cm-deep lawn edging to prevent nutrient leakage to the plants of the low nutrients group (Fig. 1). The unfertilized plants of each block were also grouped and enclosed by the same lawn edging.

Flower buds on flowering stalks were removed three times each month throughout the three years of the experiment. Half of the plants received this treatment; the other half was allowed to flower and set seeds naturally.

Measurements

In addition to the monitoring of the survival of the target plants, plant size was measured at the end of each of the three growing seasons. Leaves, rosettes, stems, and flower heads were counted; stem height, maximum leaf length and width measured. Random flower heads were bagged after flowering throughout the experiment to estimate average weight of the sexual reproductive tissue: flower head, flowers, and seeds. Total flower head weight per plant was calculated by multiplying the average of each species and treatment combination with the flower head count of the individual. In September 2002 all plants were harvested. Belowground parts were harvested in a circular area of 40 cm diameter around the center of the *M. caerulea* hexagon. The plots were dug out at least 25 cm deep, and only a very small percentage of the fine roots was lost as the plants rooted shallowly in the sandy soil. The roots of the target plant and the grass were relatively easy to separate since *M. caerulea* has thick roots (Taylor et al. 2001). The stems, leaves, caudex, roots and rhizomes of the four perennials and the grass were dried at 70°C for at least 48 hours and weighed.

Analysis

Treatment effects on the survival of the plants were analyzed with a Kaplan-Meier Log Rank test (Lee & Wang 2003) per species with bud removal and nutrient enrichment

as explaining factors in different tests. Prior to statistical analysis the number of rosettes, flowers and buds were In-transformed to improve normality. Dry weights of plants and plant parts were In-transformed when necessary to increase the homogeneity of the variance of the tested groups. Type III ANOVAs were performed on dry weights of plant parts with bud removal, fertilization and their interaction as fixed factors and plant pair nested within the fertilization treatments as random factor. Repeated Measures MANOVAs with the same factors and year as time factor were used for the data on flower and rosette numbers. Type I ANCOVAs (Zar 1996) with vegetative biomass (roots, leaves and stems) as covariate were performed to test for

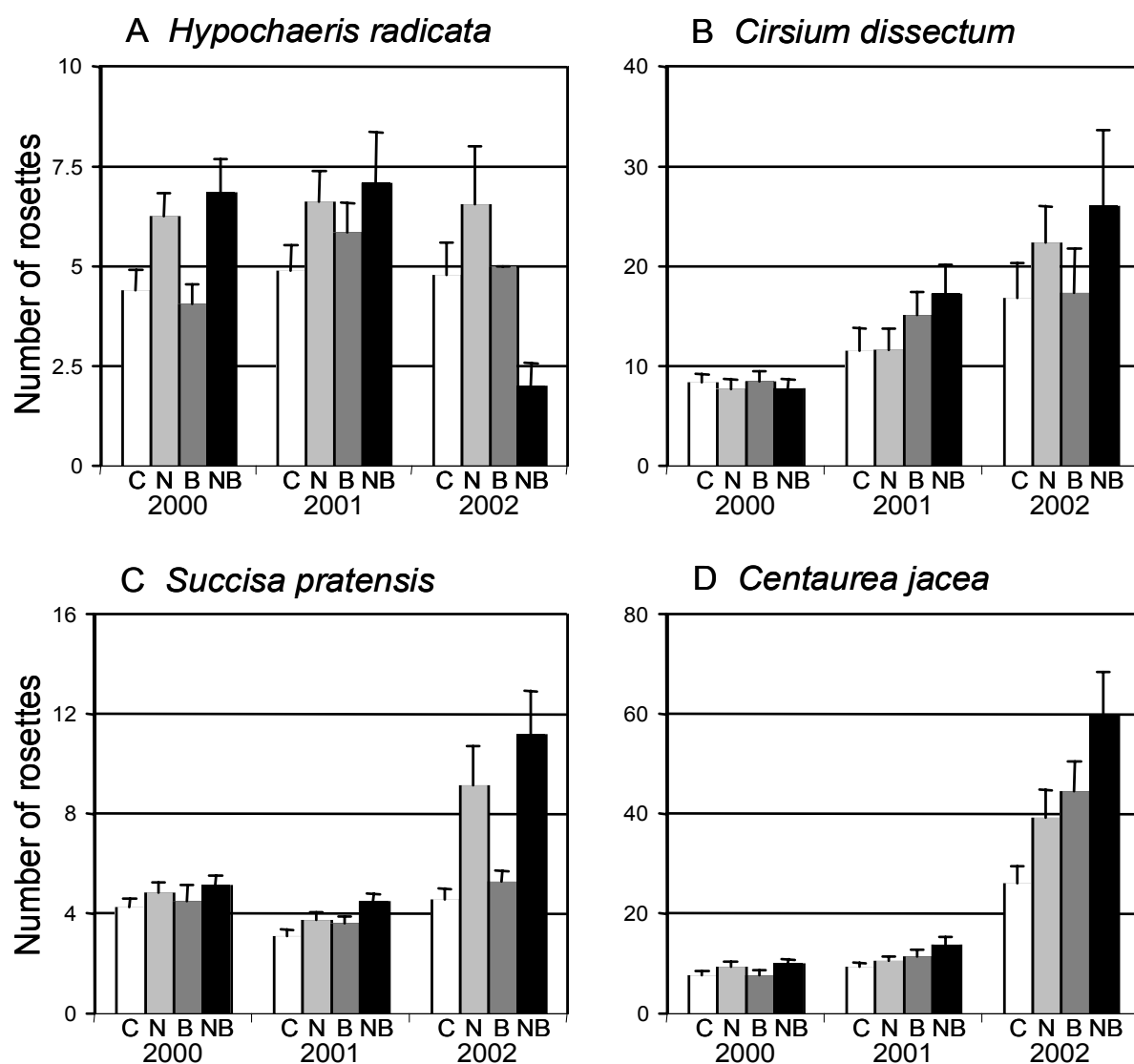


Figure 4.

Development of the number of rosettes (mean + standard error) of surviving plants. The extra nutrient treatment started June 2001. Flower buds were removed all three years. C=control. B=bud removal; N=nutrients added; NB=both bud removal as nutrients added.

differential biomass allocation to storage organs (caudex) or sexual reproduction (flower heads and seeds). Bud removal and fertilization were the fixed factors in these ANCOVAs. Plant pair was not included in these models because the plant pairs were designed to differ in plant size, and these two parameters indeed turn out to be collinear in three species (Table 1).

Results

Costs of sexual reproduction

Both total biomass and rosette formation were enhanced by continuous bud removal (Figs. 3 and 4), thus clearly showing costs of sexual reproduction for plant growth and clonal propagation. Significant costs of sexual reproduction were found in all species, although not in all years (Table 1 and 2). In *Cirsium dissectum* and *Centaurea jacea*, the average total dry weight at harvest after three years was significantly higher in the bud removal group (Table 1). The number of *Hypochaeris radicata* rosettes did not increase after the first year, and was positively affected by flower bud removal in the first two years (Fig. 4A). In *Succisa pratensis* bud removal caused a large increase (10 vs. 4.9) in rosette number in the third year (Fig. 4C), whereas rosette number was very constant in time in the unmanipulated plants. The rhizomatous *C. dissectum* increased its rosette number linearly in time (Fig. 4B). Only in the last year bud removal resulted in the formation of additional rosettes. Apart from size increases bud removal also resulted in a small increase in the relative biomass allocation to the storage organs in *S. pratensis* and *C. jacea*, but not in the other two species with shorter-lived rosettes.

Besides these switches to other life history functions, bud removal also resulted in efforts to compensate for the lost flower buds. The number of flower heads and buds of flower heads increased strongly when buds of flower heads were continuously removed in *H. radicata*, *S. pratensis* and *C. jacea*, although this effect differed between years (Fig. 5; Table 2). This compensation effect was almost absent however in *C. dissectum* (Fig. 5B), probably because it has preformed flower heads and only a short flowering period in June.

Effects of nutrient enrichment: increases in sexual reproductive effort

The total biomass of *Molinia caerulea* tripled in response to nutrient addition (91 vs. 279 g, $n=320$, $F=1.47 \cdot 10^4$, $p<0.001$). While bud removal did not influence survival, nutrient addition decreased survival in *C. dissectum* and *H. radicata* (Log Rank = 10.50, $p=0.001$ and Log Rank = 25.58, $p<0.001$ respectively; Fig. 2). At harvest survival rates in the high nutrient group decreased to 33% in *C. dissectum* and 13% in *H. radicata* (90% and 65% respectively for the low nutrient group). Two *C. jacea*

plants died and all *S. pratensis* plants survived. Only these two species were able to build up significantly more biomass when extra nutrients were given (Table 1). The effects of nutrient enrichment and bud removal on total biomass were additive and these treatments did not interact. Especially in *S. pratensis* the variance in total weight was largest in the group of plants that were fertilized as well as having their buds removed (Fig. 3), indicating that not all individuals were able to increase in size to prevent being dominated by the grasses. Within the group of plants of which no buds were removed, nutrient enrichment lowered the root-shoot ratio significantly ($p < 0.002$ in all species when testing differences in mean of the ln-transformed ratio of root biomass by stem and leaf biomass).

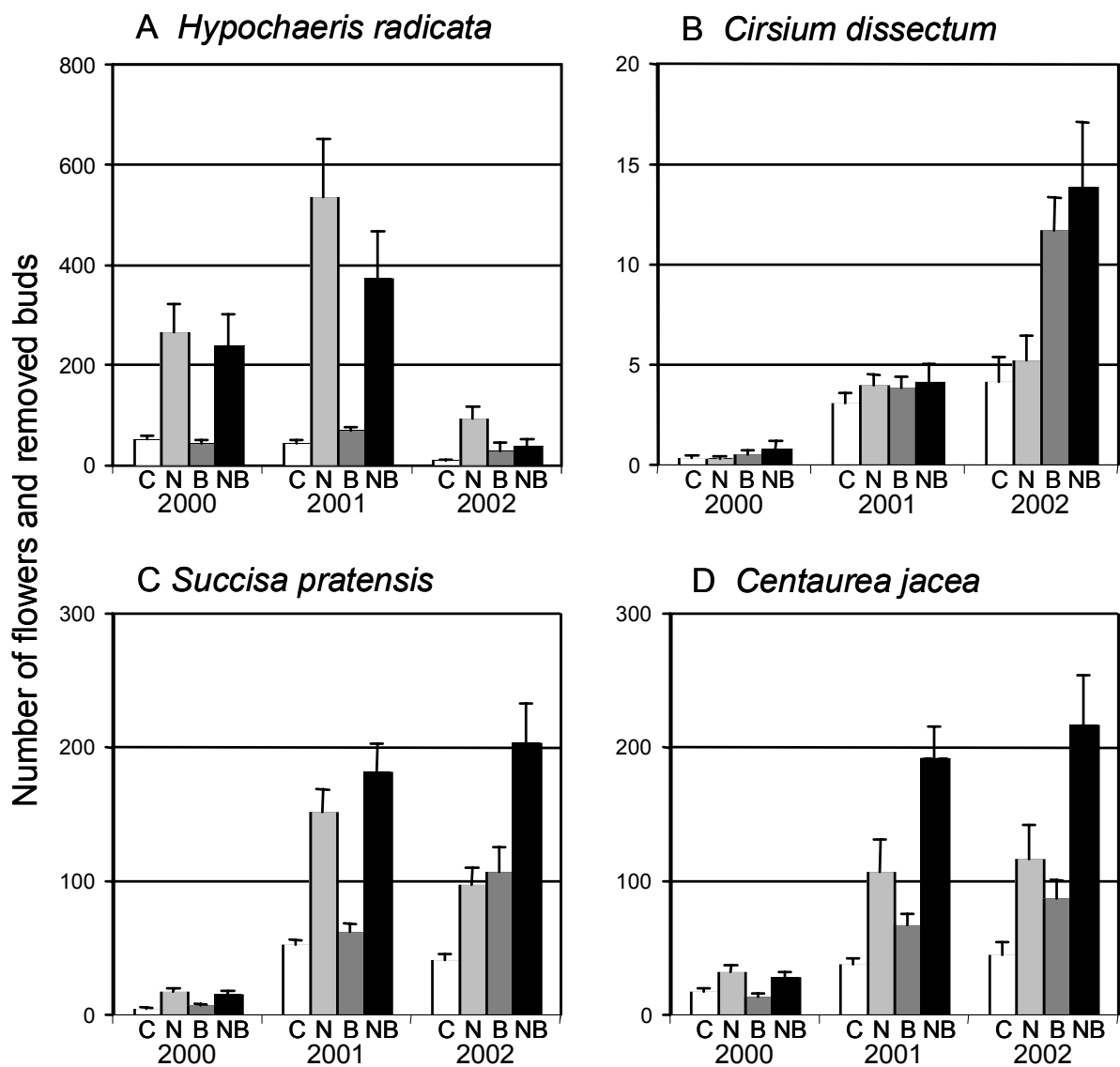


Figure 5.

Development of the number of flowers and removed buds (mean + standard error) of surviving plants. The extra nutrient treatment started June 2001. Flower buds were removed all three years. C=control. B=bud removal; N=nutrients added; N+B=both bud removal as nutrients added..

The plant in the fertilized plots allocated relatively more to sexual reproduction, while the relative allocation to storage did not change much. Thus there is little support for the persistence hypothesis as formulated in the introduction, while the results on sexual reproductive effort tend to support the escape hypothesis. Nutrient addition increased average flower number in all species but *H. radicata* (Table 2; Fig. 5). Both sexual reproductive and storage biomass were highly significantly correlated with vegetative biomass in all species (Figs. 6 and 7; Table 3). While controlling for size, nutrient addition increased sexual reproductive biomass in *H. radicata*, *C. dissectum* and *S. pratensis*. (Figs. 7A, 7B and 7C). By contrast, the slopes of the regression lines of the two *C. jacea* groups differ significantly (Fig. 7D), indicating that the nutrient addition effect was different for larger plants as compared to smaller plants. Relative allocation to storage biomass was only affected by nutrient addition in *C. jacea*, in which species storage biomass increased slightly per unit of vegetative weight (Fig. 6D).

Discussion

In our experiment we explicitly tested the reformulated hypotheses of Ogden (1974) and Abrahamson (1980) that with progressing succession plants may increase their allocation to sexual reproduction (escape strategy) or try to persist by increasing the allocation to storage and vegetative plant parts at the expense of allocation to sexual reproduction (persistence strategy). During succession nutrients accumulate in the vegetation and subordinate species are gradually driven to local extinction. We successfully mimicked this essential process of successional change by adding nutrients to grassland perennials that were grown in between tussocks of *Molinia caerulea*. As expected this tall grass increased in biomass after nutrient enrichment and caused more intense competition pressure on the target plants, resulting in high mortality rates in the short-lived *Hypochaeris radicata* and *Cirsium dissectum*. These two species have a relatively high turnover of leaf biomass, which is disadvantageous when competing with a grass species that accumulates biomass like *M. caerulea* (Berendse et al. 1987; de Kroon & Bobbink 1997; van der Krift & Berendse 2002). Mortality rates in *C. dissectum* were lower than in *H. radicata*, because this rhizome forming plant could run and escape from the increasingly dense tussocks. In the other two species, *Succisa pratensis* and *Centaurea jacea*, larger plants were able to grow larger and to secure their place in the vegetation. Only these large plants were able to keep *M. caerulea* under control and could benefit from the extra nutrients themselves. This is in agreement with Swiss field observations: with increasing site productivity *S. pratensis* density decreased, but plant size and seed production increased (Billeter et al. 2003).

One of the hypothesized phenotypic allocation responses to successional replacement was found in three out of four species, i.e. an increase in sexual

reproductive allocation. In the fourth species, *C. jacea*, the treatment effects interacted with size: larger plants had higher reproductive allocation, but smaller plants had lower reproductive allocation than unfertilized controls. In contrast, in all four species, the size dependent allocation to storage organs was hardly affected, which suggests that no costs are involved in elevated sexual reproduction under nutrient addition.

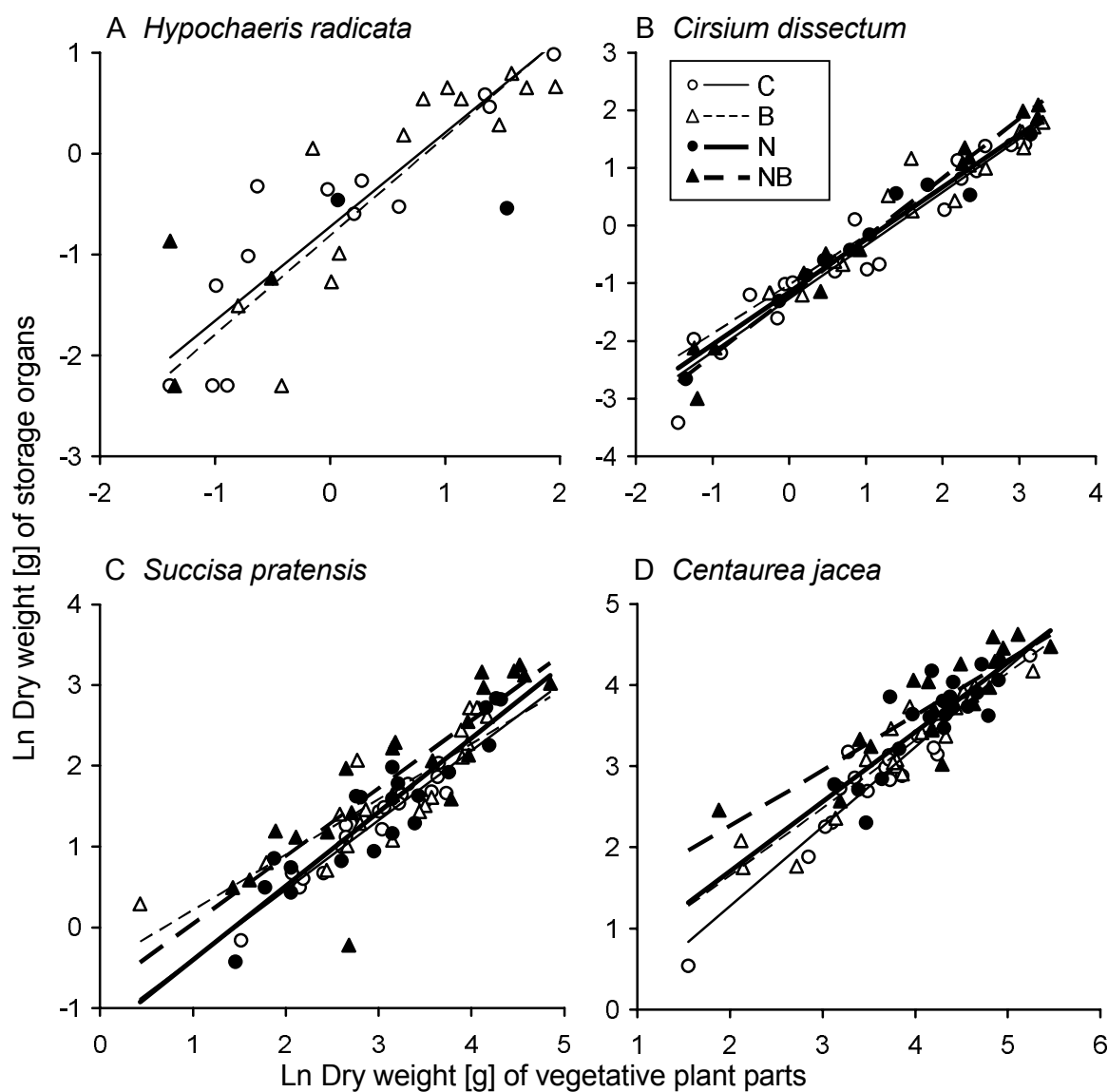


Figure 6.

Dry weight [g] of the storage organs (caudex) plotted per plant against its vegetative (roots, leaves and stems) dry weight [g] at harvest. C=control. B=bud removal; N=nutrients added; N+B=both bud removal as nutrients added.

Costs of Sexual Reproduction after three Years of Bud Removal

Sexual reproduction however does have demographic costs in the long run in long-lived perennials, as is exemplified by our results of three years of continuous flower bud removal. Inhibition of flower and seed production increased total biomass especially in *C. dissectum* and *C. jacea*, and increased rosette number especially in *S. pratensis* and *C. jacea*. In *S. pratensis* bud removal not only caused increases in total biomass but also caused meristemic responses: the number of rosettes increased relatively more than total biomass, probably due to a release of apical dominance of flowering over rosette formation. Simultaneously allocation to storage increased only slightly. Although the method of flower bud removal also induced compensation responses by activation and production of new flower buds. In spite of this additional investment in new flower buds, the method succeeded in revealing costs of sexual reproduction as it has in other studies (Avila-Sakar et al. 2001; Ehrlén & van Groenendael 2001; Obeso 2002; Hartemink et al. 2004).

Both biomass and meristemic responses to flowering inhibition eventually resulted in larger plants, which have higher survival probabilities in the field (Chapter 5). These demographic trade-offs between sexual reproduction and vegetative growth and survival indicate that it is indeed meaningful to test the hypotheses of Ogden (1974) and Abrahamson (1980) in these perennial herbs by studying shifts in relative sexual reproductive allocation in relation to other life history functions.

Sexual reproductive allocation increases in response to the nutrient treatment and is strongly size-dependent

Our experiment revealed increased seed production per plant under mimicked succession due to two factors: an increase in plant size and an increase in allocation to sexual reproduction. Three of our four species increased their relative sexual allocation irrespective of plant size after nutrient addition, which is consistent with the escape strategy. The similar responses of these species suggest that the differences between their life histories (Hartemink et al. 2004) do not matter too much for their strategy to deal with succession. Perhaps herbs need the ability to form both long runners and long-lived plant parts before they can adopt the persistence strategy.

Although the experiment was not set-up to elucidate the mechanisms behind the allocation responses, we could speculate that the increased seed production was the result of a lower root-shoot ratio under nutrient enriched conditions: seed production may have been increased because allocation to all above-ground tissues increased in comparison to allocation to roots, when the plots were fertilized. Cheplick (2001) for instance, found a strong correlation between seed output and stem mass in the annual *Amaranthus albus*. Lower costs of seed production under high nitrogen availability could be another explanation for increased sexual reproductive allocation (Saxena & Ramakrishnan 1984; Loehle 1987; Reekie 1991), but there were no interactions between nutrient addition and bud removal in our experiment which would

be expected in case nutrient addition would decrease the costs of seeds. Nor can the shift towards sexual reproductive allocation be attributed to selection of surviving plants in the two species with high mortality in the high nutrient group: on the contrary, plants with higher seed production at the expense of vegetative biomass are rather expected to be selected against when only surviving plants are analyzed.

It is remarkable that no cost of the elevated reproduction was found in the fertilized plants, while the bud removal treatment showed costs of sexual reproduction

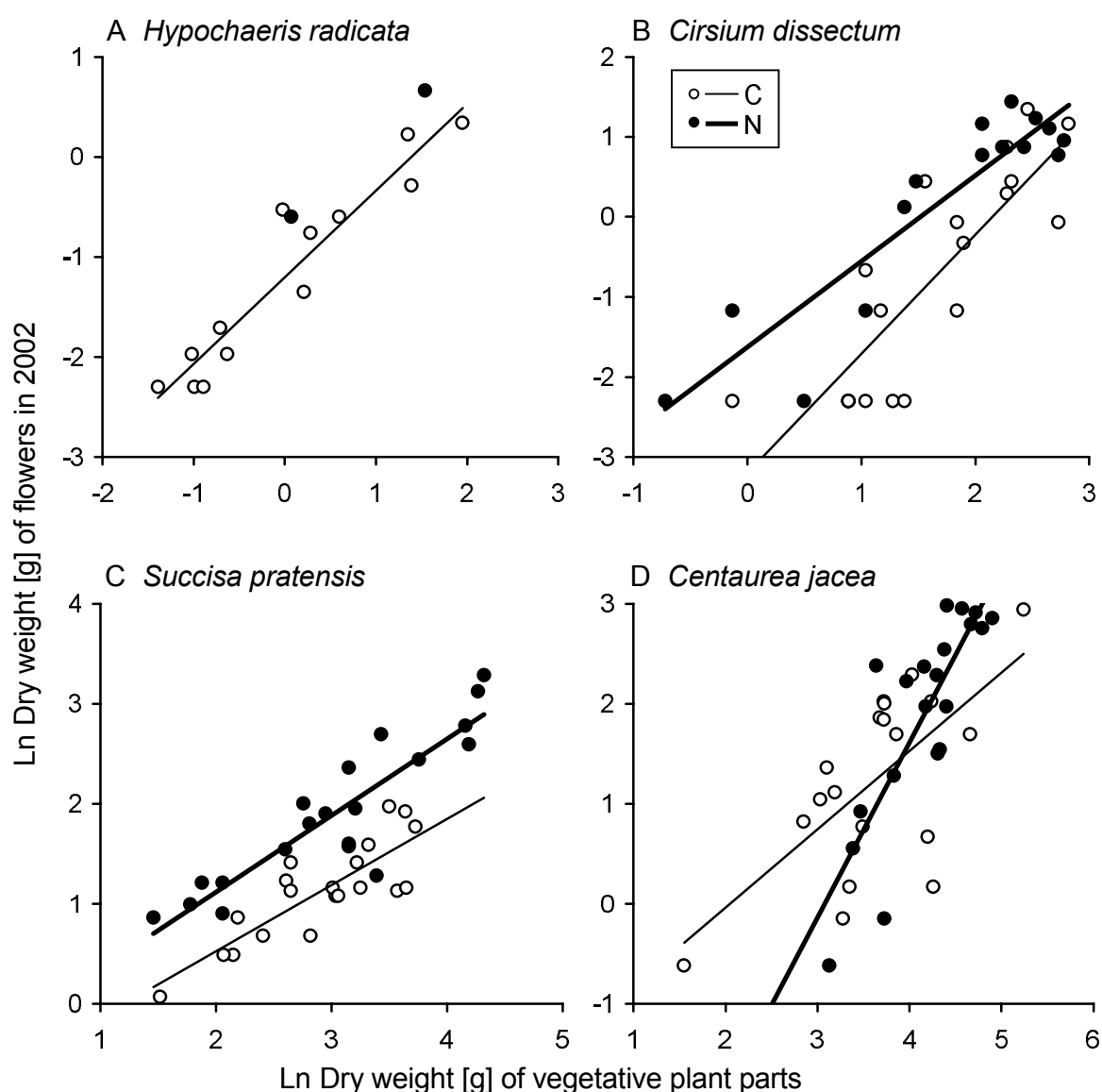


Figure 7.

Dry weight [g] of the flowers (flower heads and seeds) plotted per plant against its vegetative (roots, leaves and stems) dry weight [g] at harvest. C=control. N=nutrients added. Vegetative weight of *C. dissectum* was set back to the moment of flowering by taking the number of rosettes during flowering and multiplying it with the average rosette weight at harvest.

Table 1.

ANOVA's on dry weight (W) [g] at harvest of *Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea* with bud removal, nutrient addition and pair of plants as explaining factors. Data were ln-transformed when necessary (ln(W)). 'Flowers' = flowers, seeds and removed buds; 'Vegetative' = stems, leaves and roots; 'Storage' = caudex; df = degrees of freedom; F = ANOVA statistic; (*) = $p < 0.10$; * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$

Dry Weight	Effect	<i>H. radicata</i>		<i>C. dissectum</i>		<i>S. pratensis</i>		<i>C. jacea</i>	
		df	F	df	F	df	F	df	F
Total		W		W		ln(W)		W	
	Intercept	1	19.8 **	1	42.6 ***	1	3153.3 ***	1	397.1 ***
	Pair (within N)	20	1.2	33	1.7 (*)	38	3.7 ***	38	2.7 **
	Bud removal (B)	1	1.0	1	4.5 *	1	1.8	1	4.7 *
	Nutrients (N)	1	0.6	1	0.8	1	3.3 (*)	1	20.9 ***
	B * N	0	-	1	0.9	1	0.1	1	0.1
	Error	7	MS=7.98	22	MS=116.60	38	MS=0.30	36	MS=2257.95
Flowers		W		W		ln(W)		ln(W)	
	Intercept	1	22.4 **	1	64.4 ***	1	210.6 ***	1	160.0 ***
	Pair (within N)	20	0.9	33	1.0	38	2.6 **	38	2.4 **
	Bud removal (B)	1	0.1	1	8.8 **	1	106.8 ***	1	16.1 ***
	Nutrients (N)	1	1.8	1	13.0 **	1	36.5 ***	1	18.3 ***
	B * N	0	-	1	1.3	1	0.1	1	0.0
	Error	7	MS=0.22	22	MS=0.86	37	MS=0.30	36	MS=0.63
Vegetative		W		W		ln(W)		W	
	Intercept	1	17.0 **	1	37.1 **	1	2227.2 ***	1	350.7 ***
	Pair (within N)	20	1.2	33	1.7	38	3.5 ***	38	2.9 **
	Bud removal (B)	1	0.9	1	4.8 *	1	3.9 (*)	1	5.2 *
	Nutrients (N)	1	0.7	1	1.7	1	1.0	1	13.4 **
	B * N	0	-	1	0.8	1	0.0	1	0.1
	Error	7	MS=3.52	22	MS=51.04	38	MS=0.34	36	MS=983.96
Storage		W		ln(W)		ln(W)		ln(W)	
	Intercept	1	17.9 **	1	4.0 (*)	1	901.4 ***	1	3995.9 ***
	Pair (within N)	20	1.5	33	1.8 (*)	38	4.3 ***	38	3.3 ***
	Bud removal (B)	1	2.3	1	4.6 *	1	19.2 ***	1	7.6 **
	Nutrients (N)	1	3.7 (*)	1	2.7	1	5.0 *	1	37.6 ***
	B * N	0	-	1	0.2	1	0.1	1	0.6
	Error	7	MS=0.39	22	MS=1.29	38	MS=0.23	36	MS=0.21
Rhizomes				ln(W)					
	Intercept			1	12.7 **				
	Pair (within N)			29	1.5				
	Bud removal (B)			1	3.6 (*)				
	Nutrients (N)			1	0.1				
	B * N			1	1.6				
	Error			18	MS=2.93				

in total biomass and in the number of side rosettes. The explanation for this contradiction is that the size-dependency of seed production masks trade-offs between sexual and vegetative reproduction as large plants are better in both (Sugiyama & Bazzaz 1998; Reznick et al. 2000; Worley et al. 2003). Shipley and Dion (1992) report that plant size explains most of the variation in seed output between herb species and between different habitats or successional stages. Also in our study a larger part of the variation in sexual reproduction was explained by plant size than by

the mimicked succession. The tight relationships between storage weight and the weight of the vegetative plant parts, which is consistent with the small response of storage allocation to bud removal, suggest a strong developmental link and even less opportunity for flexible allocation towards storage than towards sexual reproduction.

Consequently the hypotheses of plant responses to successional replacement (Ogden 1974; Abrahamson 1980) need a reappraisal. Our data suggests that allocation in general is strongly dependent on plant size and that the responses to succession may be either size-independent as in *H. radicata*, *C. dissectum* and *S. pratensis*, or size-dependent like in *C. jacea*. In order to produce more seeds that may establish in more favorable patches, a plant first has to survive, and it can only do so by increasing its size to avoid shading (Huber & Wiggerman 1997). Therefore the escape strategy and persistence strategy collapse into essentially one syndrome: the plants that manage to survive increased competition respond by increasing their size, while at the same time allocating an increased percentage of their biomass to sexual reproduction. Although there are costs to increased sexual reproduction the plants are still larger.

Implications

Apart from examples in annuals like *Abutilon theophrasti* (Sugiyama & Bazzaz 1997) in which sexual offspring is the only way of increasing fitness with higher resource availability, we are not aware of other findings of increased sexual reproduction in perennials under increasing competition. However, Van Zandt et al. (2003) do show that the clonal plant *Iris hexagona* responds similarly to another type of stress, salinity. Thus empirical evidence is emerging that perennial and clonal species can adjust their life history strategy to adverse growing conditions, confirming model predictions (Sakai 1995; Saikkonen et al. 1998; Gardner & Mangel 1999; Olejniczak 2003).

Increasing seed production as habitat conditions deteriorate may have important implications for a species on a regional level. Such responses would have great implications for metapopulation dynamics, in which both persistence (patch occupancy) and sexual reproduction (production of diaspores for colonization of empty patches) are key parameters (Eriksson 1996; Soons et al. 2003). Especially for a Red List-species as *C. dissectum* that only survives in a small number of remnant populations (Soons et al. 2003), seed production level can be a crucial limitation for colonization. A relative increase of seed production may be the last sign of life before a remnant population becomes a senile one in which succession advances and seedlings no longer can establish.

Table 2.

Repeated measures MANOVAs on rosette numbers and numbers of flowers or removed buds at the end of the three consecutive years of the allocation experiment with *Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*. Data were transformed ($\ln(\text{number} + 0.1)$) prior to analysis. df = degrees of freedom; F = Wilk's Lambda statistic; (*) = $p < 0.10$; * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$

		<i>H. radicata</i>		<i>C. dissectum</i>		<i>S. pratensis</i>		<i>C. jacea</i>	
Number of	Effect of	df	F	df	F	df	F	df	F
Rosettes									
<i>Between subjects</i>									
	Intercept	1	120.1 ***	1	818.5 ***	1	2612.4 ***	1	3645.0 ***
	Pair (within N)	20	1.3	33	2.9 **	38	2.7 **	38	2.9 **
	Bud removal (B)	1	0.7	1	0.8	1	31.4 ***	1	8.5 **
	Nutrients (N)	1	0.1	1	0.0	1	4.9 *	1	6.3 *
	B * N	0	-	1	0.0	1	0.2	1	0.0
	Error	7	MS=0.87	22	MS=0.87	38	MS=0.21	36	MS=0.42
<i>Within subjects</i>									
	Year (Y)	2	2.5	2	9.4 **	2	55.6 ***	2	88.3 ***
	Y * Pair (within N)	40	6.4 **	66	1.9 *	76	1.4 (*)	76	1.4
	Y * Bud removal (B)	2	0.2	2	1.8	2	8.5 **	2	0.4
	Y * Nutrients (N)	2	1.8	2	12.7 ***	2	1.4	2	3.1 (*)
	Y * B * N	0	-	2	0.3	2	0.2	2	0.4
Flowers or Removed Buds									
<i>Between subjects</i>									
	Intercept	1	420.0 ***	1	3.0 (*)	1	5074.4 ***	1	2407.9 ***
	Pair (within N)	20	2.1	33	2.2 *	38	7.1 ***	38	2.1 *
	Bud removal (B)	1	35.5 **	1	0.5	1	85.5 ***	1	40.1 ***
	Nutrients (N)	1	0.2	1	8.5 **	1	11.3 **	1	10.4 **
	B * N	0	-	1	1.1	1	0.7	1	0.0
	Error	7	MS=1.61	22	MS=1.38	38	MS=0.55	36	MS=1.25
<i>Within subjects</i>									
	Year (Y)	2	37.6 ***	2	107.9 ***	2	560.3 ***	2	80.0 ***
	Y * Pair (within N)	40	2.4 (*)	66	1.2	76	4.1 ***	76	1.3
	Y * Bud removal (B)	2	6.0 *	2	1.2	2	4.7 *	2	0.4
	Y * Nutrients (N)	2	4.0 (*)	2	3.7 *	2	11.5 ***	2	5.4 **
	Y * B * N	0	-	2	0.2	2	2.1	2	0.8

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Table 3.

ANCOVAs per species (*Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*) on the dry weight [g] of the storage organs (caudex) at harvest with bud removal and nutrient addition as fixed factors and vegetative dry weight (roots, leaves and stems) as covariate. The dry weights of the flowers in 2002 of the undisturbed (no bud removal) plants were analyzed the same way. The data on dry weight were ln-transformed. df = degrees of freedom; F = ANCOVA statistic; (*) = $p < 0.10$; * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$

Dry Weight		<i>H. radicata</i>		<i>C. dissectum</i>		<i>S. pratensis</i>		<i>C. jacea</i>	
Effect		df	F	df	F	df	F	df	F
<i>Storage organs</i>									
Intercept		1	27.9 ***	1	409.2 ***	1	1617.2 ***	1	10780.7 ***
Vegetative Weight (V)		1	77.5 ***	1	854.1 ***	1	318.5 ***	1	433.8 ***
Bud removal (B)		1	0.2	1	0.6	1	10.6 **	1	4.3 *
Nutrients (N)		1	0.1	1	0.2	1	3.0 (*)	1	13.9 ***
B * N		1	1.4	1	1.9	1	0.1	1	0.5
B * V		1	0.1	1	0.1	1	1.3	1	3.7 (*)
N * V		1	3.6 (*)	1	1.6	1	1.5	1	2.2
B * N * V		1	0.1	1	2.2	1	0.2	1	0.0
Error		23	MS=0.31	51	MS=0.10	72	MS=0.13	70	MS=0.082
<i>Flowers</i>									
Intercept		1	166.5 ***	1	4.6 *	1	958.9 ***	1	223.1 ***
Vegetative Weight (V)		1	138.8 ***	1	95.9 ***	1	134.0 ***	1	50.1 ***
Nutrients (N)		1	4.9 *	1	6.2 *	1	49.5 ***	1	0.2
V * N		1	0.0	1	2.5	1	0.6	1	7.0 *
Error		11	MS=0.095	25	MS=0.41	36	MS=0.096	35	MS=0.44

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Bottlenecks and spatiotemporal variation in the sexual reproduction pathway of perennials in meadows

Eelke Jongejans, Merel B Soons & Hans de Kroon

Summary

Sexual reproduction is important for population expansion and maintenance of genetic diversity. Several steps are involved in the sexual reproduction pathway of plants: production of flowers, production of seeds and establishment of seedlings from seeds. In this paper we quantify the relative importance and spatiotemporal variability of the different steps involved. The grassland perennials studied were *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 number of flower heads per flowering rosette, flower and seed number per flower head, and 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 spatiotemporal 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 determine year-to-year

population dynamics. Many studies indicate that sexual reproduction in long-lived plants is relatively less important for population growth than adult survival (Eriksson 1989; Picó & Riba 2002), but sexual reproduction can markedly increase population growth rate in some cases (Silvertown et al. 1993; de Kroon et al. 2000). In addition, sexual reproduction has other merits 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).

Although important in both a local and a regional perspective, sexual reproduction 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 consists of the production of seeds by flowering plants, and establishment of these seeds as seedlings and finally as reproductive adults. This involves several different processes: production of flower heads and flowers, pollination of flowers, production of viable seeds, arrival of seeds at microsites that are suitable for both germination and establishment, and subsequently the survival of seedlings (Fig. 1). Separately, these aspects have been illuminated by several studies. Research on seed production has focused for instance on effects of population size (Oostermeijer et al. 1998; Brys et al. 2004), geographic distribution (Jump & Woodward 2003), 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 seedling establishment in *Cirsium canescens* (Louda & Potvin 1995). Research on seedling establishment has mainly focused on the central 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 a moss layer, and the creation of gaps of bare soil in the vegetation can enhance seedling numbers (Špacková et al. 1998; Kotorová & Lepš 1999; Isselstein et al. 2002).

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

Although such studies are more common in forest ecology (Schupp 1990), the number of studies that subsequently 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 to identify the bottlenecks, and compare the temporal and spatial variation in all steps. As a study system we use four perennial plant species that co-occur in nutrient-poor meadows in the Netherlands (Soons et al. 2003; Hartemink et al. 2004). Specifically we address the following questions: What are the

bottlenecks in the sexual reproduction pathway of four perennials in meadows? Which steps have the highest temporal and spatial coefficients of variation and are important for the predicted variation in seedling numbers?

Materials and methods

Study system

We compared four perennials of nutrient poor meadows and mown road verges: the three Asteraceae species *Hypochaeris radicata* L., *Cirsium dissectum* (L.) Hill and

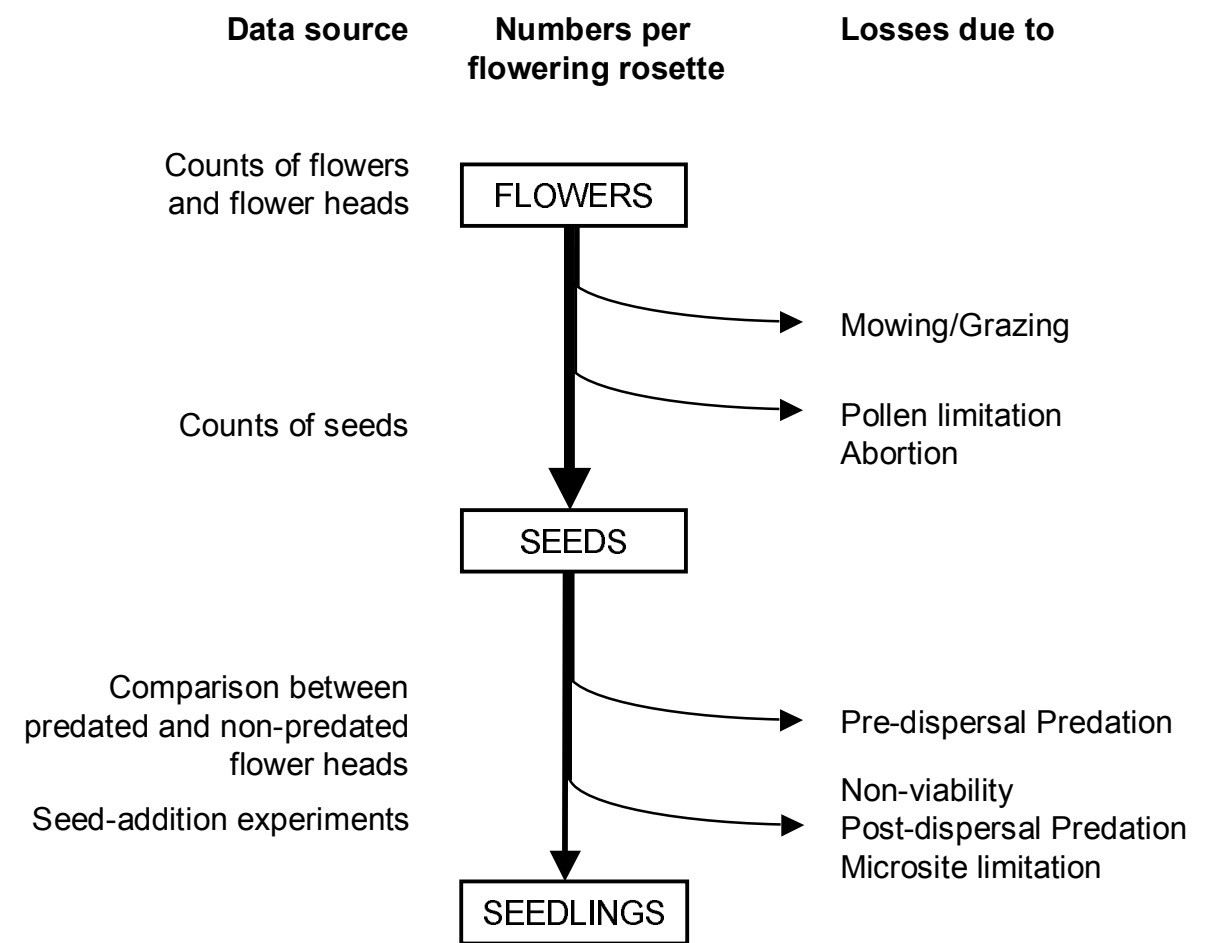


Figure 1. Schematic overview of the sexual reproduction pathway from flower production to seed set to seedling establishment. Causes of losses of ovules (right) and data sources in this study on the different parts of the pathway (left) are indicated.

Centaurea jacea L. s.l. and the Dipsacaceous species *Succisa pratensis* Moench. *Hypochaeris radicata* is short-lived (seldom 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. All four species produce flowers in flower heads with potentially one seed per flower. The seeds have similar weight, but those of *H. radicata* and *C. dissectum* have pappus that may enhance dispersal by wind (Jongejans & Schippers 1999; Soons & Heil 2002; Mix et al. 2003).

The study sites are in the central-eastern part of the Netherlands and are clustered in four regions. The range of sites includes nutrient-poor, undisturbed meadows and restoration areas where the top soil layer is removed. A list of all sites and their codes can be found in Table 1 on page 58.

Flower head production

Flowering was monitored in three to eight permanent plots of 1 m² in 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.

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 viability, that no seeds had disseminated and that in 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 strong 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 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 were predated or not. Signs of predation were: crumbled or partly eaten seeds, and the presence of insect larvae. The impact of predation on a population was evaluated by comparing the mean seed-flower ratio of non-predated flower heads with the mean ratio of all flower heads.

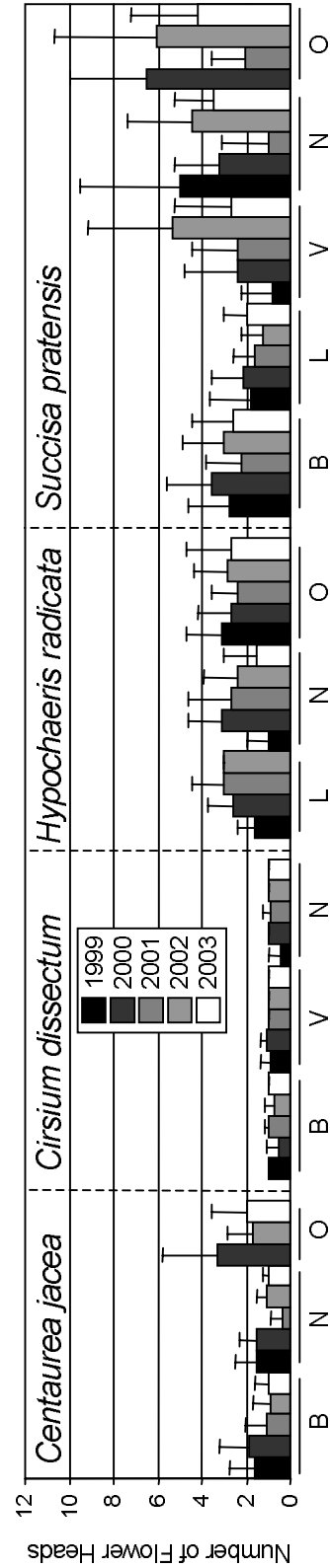


Figure 2. The average number of flower heads per flowering rosette in permanent plots per species, site, and year. Error bars denote standard deviations. See table 1 on page 60 for site codes.

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 similar sized control plots (no seeds added). In each plot 100 seeds of one 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. 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 layed 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 ten sites: seven undisturbed meadows and three meadows where the top soil was removed by sod-cutting (this second experiment is also described by Soons et al. (2003)). 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 20 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 these steps, we calculated the average number of seedlings produced by a single flowering rosette. To do this, we multiplied the consecutive steps of the sexual reproduction pathway: the mean number of flower heads per flowering rosette, the mean number of flowers per flower head, the seed-flower ratio, the fraction of seeds that are not predated and the average fraction of seeds that establish 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 result of the whole sequence of successive steps was determined using the following equation for the variance of the product of independent variables (Goodman 1960):

$$\text{var}(\bar{xy}) = \text{var}(\bar{x})\text{var}(\bar{y}) + \bar{x}^2\text{var}(\bar{y}) + \bar{y}^2\text{var}(\bar{x}) \quad (1)$$

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

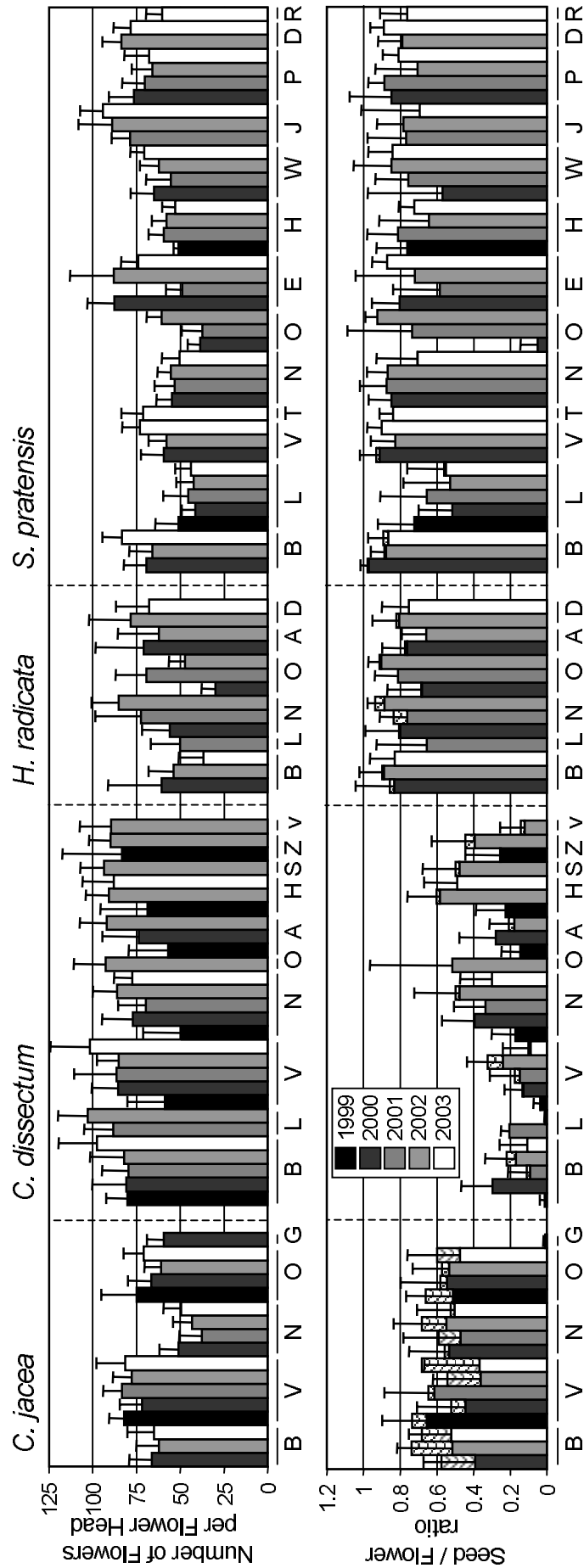


Figure 3. The average number of flowers per flower head and the seed-flower ratio of top flower heads collected in different sites (see Table 1 on page 60 for site codes) and years, of plants of *Centaurea jacea*, *Cirsium dissectum*, *Hypochaeris radicata* and *Succisa pratensis*. Error bars denote standard deviations. The level of the average seed-flower ratio without predated flower heads, is marked by the hatched bars on top.

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:

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

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

in which for each species-step combination separately \bar{x}_{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, $s_{\text{temporal},m}$ the standard deviation between the year-means in site m , and $s_{\text{spatial},n}$ 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 (Fig 2. and Table 2). However, the main effects of site and year are only significant in *C. jacea*, in which 2000 was a year with many flower heads.

Flower production and seed set

On average *C. dissectum* had significantly larger number of flowers per flower head than the other three species, and *C. jacea* more than *S. pratensis*. *Hypochaeris radicata* was intermediate to the last two species and differed only from *C. dissectum* (Fig. 3). For each species, site-year interactions were significant (Table 2): the flower heads of *H. radicata* for instance had few flowers in site O in 2000, but higher numbers in other sites that year or in other years in that site. In *C. dissectum* there was a main effect of year: in 1999 flower heads contained smaller number of flowers than in other years. In *C. jacea* and *S. pratensis* 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 *H. radicata* (Table 2). Seed predation significantly reduced seed-flower ratios in all species, but especially in *C. jacea*. In that species insect larvae regularly consume a

large part of the developing seeds. Significant site-year interactions and main effects of sites were present in most species. The factor year was only significant in *C. dissectum*: 1999 was also a bad year for seed set in this species.

Seedling establishment

Seedling establishment was highest in *S. pratensis* and *H. radicata* (Fig. 4). The first seed addition experiment, which was restricted to meadows, revealed significant effects of seed addition only in these two species (Table 2). In *C. jacea* and *C. dissectum* changes in rosette number in seeded plots were not statistically different from the control plots. The second experiment, which also included meadows of which the top soil was removed, shows seed addition effects for all species. In the very open habitats (top soil removed) establishment is higher than in undisturbed meadows for *C. jacea* and *H. radicata* and slightly so in *C. dissectum*, but not in *S. pratensis*.

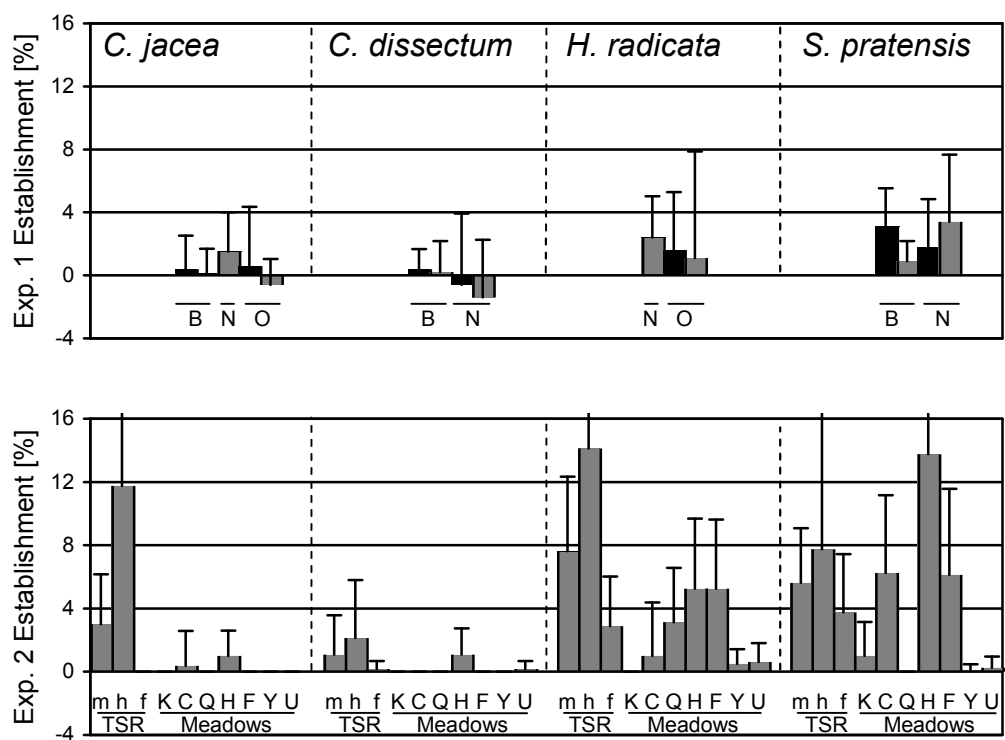


Figure 4.

The average percentage of sown seeds that is established as seedling after 12 months per species, sites, and years. Error bars denote standard deviations. The species are *Centaurea jacea*, *Cirsium dissectum*, *Hypochaeris radicata* and *Succisa pratensis*. The first experiment in the meadows B, N, and O was started in 1999 (black) and 2000 (dark grey). Since no local seed were available *C. jacea* and *H. radicata* were not seeded in site N in 1999. In the second experiment seeds were only sown in 2000 in meadows with the top soil removed (TSR), and in undisturbed meadows. Please note that negative values can occur when the change in rosette number was more positive in the control plots than in the seed addition plots. For site codes see Table 1 on page 60.

When the different steps of the sexual reproduction pathway are compared quantitatively (Fig. 5), the largest losses take place in the seedling establishment step. Beside 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 1 (respectively 1.9 and 3.3), indicating that each flowering rosette would produce one or more new rosettes via seed on a yearly basis. In *C. jacea* however the calculated seedling number was lower than 1 (0.23), and in *C. dissectum* even 0.027.

The temporal and spatial coefficients of variation (CVs) show 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. 4). The highest CVs were those of the spatial differences in seedling establishment. Although they are 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. Apart from temporal and spatial aspects of seedling establishment, relatively high variation occurred in the number of flower heads per flowering rosette in *C. jacea* and *S. pratensis* and the seed-flower ratio 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 cases such as seed set in *C. dissectum*. We found the lowest recruitment rate in *C. dissectum* and *C. jacea* which both show extensive clonal growth. We calculated that every flowering rosette has a chance of producing a seedling of 1 to 4 (*C. jacea*) or 1 to 38 (*C. dissectum*) per year. In the latter species this probability is still less than one when the highest population means for each step were used. However, clonal species need less recruitment per year to maintain population size and, more importantly, to maintain genetic diversity (Eriksson 1989; Cain 1990; Watkinson & Powell 1993). Besides, a genetic 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 recombination. It is also remarkable that, when comparing species-average sexual reproduction pathways (Fig. 5), *S. pratensis* and *H. radicata* have very similar patterns, whereas 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 for a

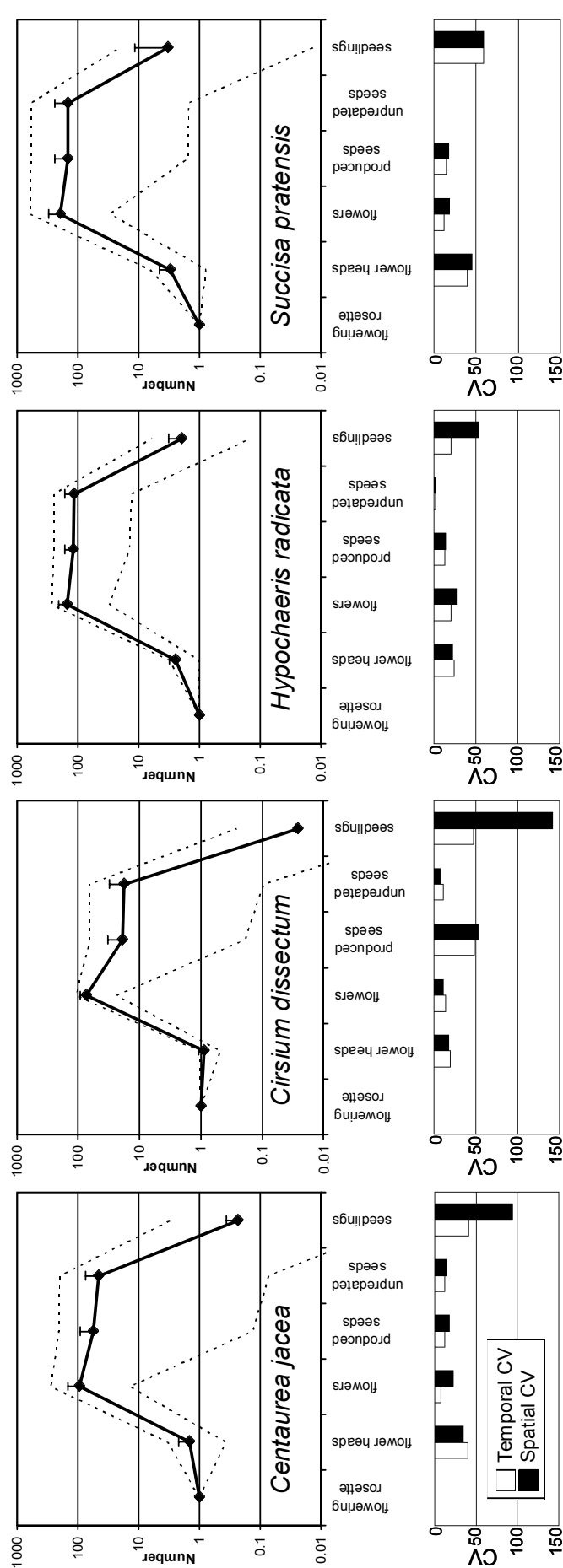


Figure 5. Above: The average (solid line) sexual reproduction pathway per species (above), based on the means of all available site-year combinations. The error bars are progressing standard deviations (see methods), and the dashed lines represent scenarios in which all steps are either the lowest or the highest observed site-year mean. The low scenario in *C. jacea* and *C. dissectum* end with zero seedlings. Below: The spatial and temporal coefficient of variance of each step is given per species (see materials and methods).

Table 1.

Code, name, and type of the sites that were used in this study. Per region coordinates are given. Meadows are mown annually, road verges once or twice per year. Sites 'm' and 'f' were sod-cut in the winter of 1999/2000, 'h' in the winter of 1997/1998.

Region	Code	Name	Type
Gelderse Vallei (52°01' N, 5°35' E)			
	B	Bennekomse meent	meadow
	A	Allemanskampje	meadow
	Z	Groot Zandbrink	meadow
Veluwe (52°17' N, 5°44' E)			
	L	Leemputten	meadow
Kop van Overijssel (52°37' N, 6°10' E)			
	P	Olde Staphorst	meadow
	T	Stadsgaten	meadow
	V	Veerslootlanden	meadow
	D	Middenweg	road verge
	v	Veerslootlanden	top soil removed
Achterhoek (51°59' N, 6°25' E)			
	C	Koolmansdijk (more productive part)	meadow
	F	Stuifveen	meadow
	H	Nijkampse heide	meadow
	K	Heksengat	meadow
	N	Konijnendijk	meadow
	O	Koolmansdijk	meadow
	Q	Koolmansdijk (heather)	meadow
	R	Breedslatsweg	meadow
	S	Stelkampsveld	meadow
	U	Vildersveen	meadow
	Y	't Veller	meadow
	E	Eibergseweg	road verge
	G	Kooigootsweg	road verge
	J	Nijkampseweg	road verge
	W	Sonderenweg	road verge
	f	Stuifveen	top soil removed
	h	Nijkampse heide	top soil removed
	m	De Maandag	top soil removed

small part by inviability of intact seeds that were used for the seed addition experiments. Soons and Heil (2002) report germination percentages for the same four species to be lower than 50% only in *S. pratensis* (for which species they found 20% germination). Other explanations like microsite limitation and post-dispersal seed predation are therefore probably more important for explaining these low recruitment probabilities.

Kotorová and Lepš (1999) found similar patterns for *S. pratensis* as in our study, when they compared seedling recruitment between their Czech sod-cut and control sites. Top soil and vegetation removal creates opportunities for seedlings, but

also causes abiotic stress due to ammonium accumulation (de Graaf et al. 1998; Dorland et al. 2003). This effect has been shown to hamper *C. dissectum* and *S. pratensis* seedling establishment for up to a year after sod cutting (Dorland et al. 2003). This may partly explain the lack of significantly higher seedling number, although 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 as well, whereas *C. dissectum* still is expected to establish better in sod cut areas but apparently still in low numbers. *H. 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 relatively common.

Flower production per flower head largely determines the number of seeds produced 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 processes are at work in *C. dissectum* which is a very rare species in the Netherlands. Losses due to seed predation are most significant in *C. jacea* (18% of the seeds on average). In accordance with Fenner et al. (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 these in the seedling establishment step.

Seed production is not only important for within population dynamics, but seed number is also a major parameter for regional population dynamics, since it partly determines colonization capacity (Soons & Heil 2002). Considering problems caused by habitat fragmentation, but possibly also by habitat range displacements due to climate change, seed production and seed dispersal may therefore be as important as seedling establishment. Because establishment probabilities may be different outside the site of the seed source, a regional evaluation of the importance of the different steps of sexual reproduction would require modeling of both local and spatial dynamics (Higgins & Cain 2002).

Temporal and spatial variation

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

Table 2.

Statistics of type III ANOVA models per species. When reported seed addition is built in as a fixed factor, 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 seed-flower ratios arcsine transformed, and the increases in rosette number were log transformed after adding 10 (Fowler et al. 1998). (*) = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Source	<i>Centaurea jacea</i>			<i>Cirsium dissectum</i>			<i>Hypochaeris radicata</i>			<i>Succisa pratensis</i>		
	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 ***
# 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 ***
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 ***
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 **
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 (*)

Although the largest reductions took place in germination and seedling recruitment, 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 which steps of the sexual reproduction pathway are the most important play grounds for selection (Koenig et al. 2003). In our study it was again seedling establishment that had the highest within-population CVs, but temporal variability was considerable as well in seed-flower ratio in *C. dissectum* and flower head production in *C. jacea* and *S. pratensis*. High spatial CVs may further enhance local adaptation, whereas high temporal CVs may select against specialization.

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Space versus time variation in the population dynamics of *Succisa pratensis* and two accompanying species

Eelke Jongejans & Hans de Kroon

Summary

Many plant species are currently restricted to small and isolated populations due to habitat destruction and habitat fragmentation. To obtain sufficient data for management temporal variation has often been substituted with spatial variation, but it is still largely unknown whether there are substantial differences between these two types of variation. In order to fill this gap we studied the demography of the declining perennial *Succisa pratensis* at five sites over four years (1999-2003). Furthermore, to contrast with the long-lived life cycle of *S. pratensis*, two coexisting species with similar life cycle components (fecundity, survival, growth and ramification) were studied in the same plots: the shorter-lived *Hypochaeris radicata* and the long-lived, but more clonal *Centaurea jacea*. The *S. pratensis* populations showed high elasticities for adult rosette survival. Life table response experiment analysis (LTRE) revealed that temporal and spatial variation in the life history components were qualitatively different: fecundity contributed most to variation in population growth rate (λ) between sites, but growth contributed most to variation in λ between years. In bad years most of the life history components were reduced, whereas bad sites had low λ s for different reasons: all major components were reduced in the most productive site, while in two other bad sites the negative contributions to the variation in λ by some components (fecundity or growth) were partly compensated for by positive contributions by other components (growth or stasis). While the results of *H. radicata* were similar to those of *S. pratensis*, *C. jacea* showed a reverse pattern: in bad years negative contributions by some life history components were buffered by positive contributions by other components, while this buffering did not occur in bad sites. Our analysis shows that the population dynamics of perennial plants may respond differently to temporal than to spatial variation in site-specific environmental conditions. Moreover, co-occurring species with similar life history options responded differently to the same spatiotemporal variation. We conclude that temporal dynamics can not readily be exchanged with spatial dynamics in population viability analyses and management studies of a species.

Keywords: *demography, elasticity, life history components, spatial and temporal variation, three-way fixed-factor variance decomposition, trade-off*

Introduction

Many plant species that are characteristic of nutrient-poor species-rich moist grasslands are threatened by environmental changes due to habitat fragmentation and intensified land use: the water tables are lowered by drainage, and nutrient enrichment and acidification are caused by nitrogen deposition and enriched groundwater from neighbouring agricultural fields cause nutrient enrichment and acidification and the remaining populations have become smaller and more isolated (Saunders et al. 1991; Tilman et al. 1994; Roem & Berendse 2000; Hanski & Ovaskainen 2000; Lucassen et al. 2003; Oostermeijer 2003; Vergeer et al. 2003). The processes outlined above may have irreversible or at least long-term effects on these meadows (Berendse et al. 1992; Bakker & Berendse 1999; Lucassen et al. 2003; van der Hoek et al. 2004). Individual sites differ in fragmentation history, the increase in productivity, abiotic conditions, vegetation composition and management regime (Soons & Heil 2002; Soons et al. 2003; Vergeer et al. 2003). Annual mowing is a stabilizing factor as it halts succession, but differences between years do exist due to variation in mowing date and climatic factors such as rainfall. The dynamics of plant populations respond to this spatiotemporal variation in site-specific environmental conditions (Oostermeijer et al. 1996; Menges & Dolan 1998; Watkinson et al. 2000).

In order to fine-tune management for declining plant species, thorough knowledge is needed about the relative importance of different life history components (Oostermeijer et al. 1996). General patterns between species have been shown: seedling establishment contribute more to the population growth in short-lived species (see for an example Picó et al. 2003), and survival of individuals more in long-lived plants (Silvertown et al. 1993). Furthermore, within species, good years or sites are associated with increased importance of seed production. In bad years or sites plants seem to rely more on survival (Oostermeijer et al. 1996; Menges & Dolan 1998; Valverde & Silvertown 1998). In order to quickly gather data for management it is tempting to substitute temporal variation by spatial variation (Silva et al. 1991; Damman & Cain 1998; Valverde & Silvertown 1998; Bühler & Schmid 2001; Quintana-Ascencio et al. 2003). However, very few studies have investigated whether temporal and spatial variation in life history components is interchangeable (Horvitz et al. 1997). A systematic analysis of whether and how the life history components of a species covary in response to temporal or spatial variation is still lacking. This paper is an attempt to fill this gap.

Population matrix projection models provide a powerful tool for studying the importance of different life history components between sites or years. Several authors have shown that the relative importance, or elasticity, of particular matrix elements for

the projected population growth rate (λ), is correlated with λ (Silvertown et al. 1993; Oostermeijer et al. 1996; de Kroon et al. 2000). However, differences in elasticities do not need to coincide with actual changes in life history components that have caused the differences in population growth rate to occur, as elasticities are only local properties of a certain matrix (Moloney 1988; Horvitz et al. 1997). To investigate what

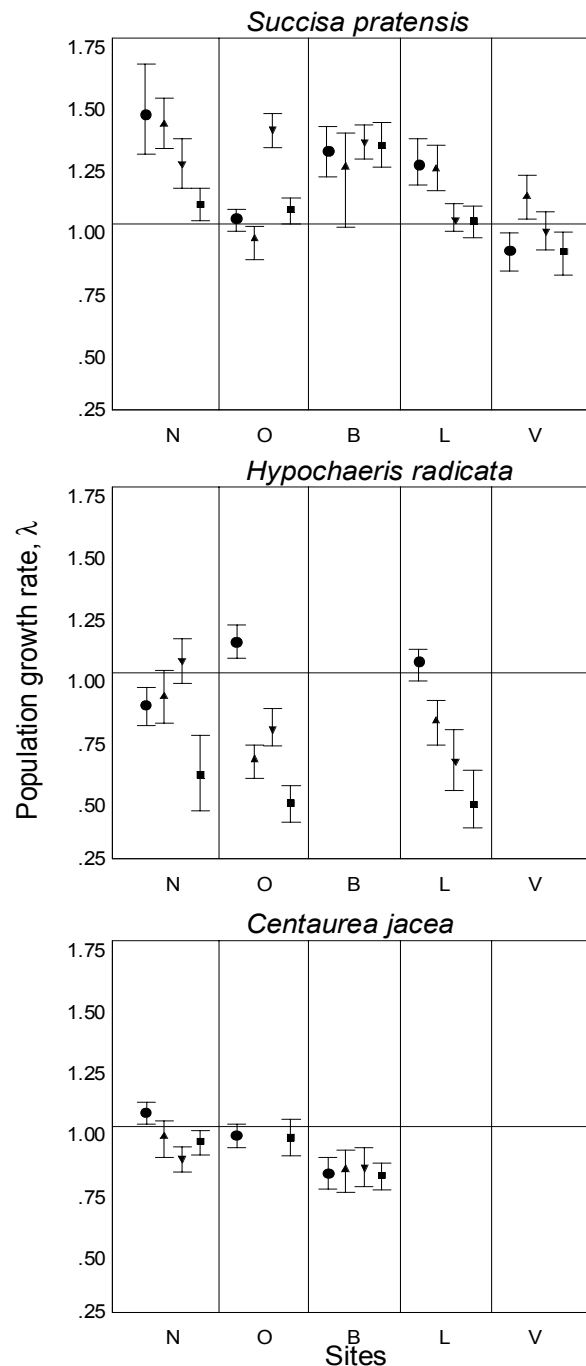


Figure 1.

Projected population growth rate, λ , and bootstrapped 95% confidence intervals for all constructed matrices. Site and year codes: N = Konijnendijk, O = Koolmansdijk, B = Bennekomse Meent, L = Leemputten, V = Veerslootlanden, ● = 1999->2000, ▲ = 2000->2001, ▼ = 2001->2002 and ■ = 2002->2003.

actually caused changes in λ , techniques have been developed that decompose the variation in λ into contributions of the underlying components (Horvitz et al. 1997; Caswell 2000).

In this paper we compare LTRE (life table response experiment, a variance decomposition technique) contributions and elasticities for an overall declining long-lived perennial herb, *Succisa pratensis*. After studying its demography in five sites for four years, we investigate whether variation in the same life history components determine the spatial and temporal variation in λ . Furthermore, in order to contrast the patterns of the long-lived *S. pratensis* with two different life history strategies, we also studied two co-occurring but less declining species: the short-lived perennial *Hypochaeris radicata* and the long-lived, clonal *Centaurea jacea*. We monitored populations of these species simultaneously with *S. pratensis* in a subset of the sites.

Materials and methods

Study system

Nutrient-poor, species-rich, moist meadows declined in abundance by more than 99% in the 20th century in the Netherlands due to drainage, cultivation, and fertilization, and are now restricted to small nature reserves (Berendse et al. 1992; Soons et al. 2003). In former days these wet grasslands were solely used for haymaking and extensive cattle grazing (Pegtel 1983; Grootjans et al. 2002). Currently conservation management is aimed at conserving local floristic diversity in two ways: annual mowing and hay removal, and restoring hydrological conditions by halting influx of nutrient-rich surface water and restoring the upwelling of base-rich groundwater.

Succisa pratensis Moench (Devil's bit Scabious, Dipsacaceae), is a long-lived perennial with polycarpic rosettes (Adams 1955; Hooftman & Diemer 2002) and characteristic of *Cirsio dissecti*-*Molinietum* communities (Schaminée et al. 1996). Leaves grow in alternating pairs and stay alive for almost a year. Flowering stalks arise from axillary buds of old leaves and flowers are produced in flower heads from late July till October. Axillary buds occasionally form side rosettes on short stolons. After one year most short stolons are severed naturally.

Hypochaeris radicata L. (Cat's ear, Asteraceae) is a relatively short-lived perennial (de Kroon et al. 1987; Fone 1989). Its flowering stalks and new rosettes are formed clonally in the centre of the main rosette; the number of leafless flowering stalks varies from one to several. Flowering starts in June and continues until autumn.

Centaurea jacea L. s.l. (Knapweed, Asteraceae), is a long-lived perennial with monocarpic shoots (Tamm 1956). *Centaurea jacea* has a single apical flowering stalk and flowers from June until autumn. During and after flowering, vegetative side-rosettes are formed on the woody rootstock at the soil surface alongside the flowering stem (Hartemink et al. 2004). *Centaurea jacea* and *H. radicata* have a wider

distribution than *S. pratensis* and also occur in more productive or disturbed grasslands and road verges.

Permanent plots

Five *S. pratensis*, three *H. radicata* and three *C. jacea* populations were studied from 1999 until 2003, resulting in 20, 12 and 10 year-to-year transition matrices for each species, respectively. All sites were annually mown, nutrient-poor grasslands in the Centre and East of the Netherlands. Arranged from low to high productivity, the sites were Leemputten (L) (52°17' N, 5°44' E), Bennekomse Meent (B) (52°00' N, 5°36' E), Konijnendijk (N) (52°02' N, 6°26' E), Koolmansdijk (O) (52°01' N, 6°33' E) and Veerslootlanden (V) (52°36' N, 6°08' E). *Hypochaeris radicata* was studied in the same plots as *S. pratensis* in sites L, N, and O, and *C. jacea* in the same plots as *S. pratensis* in sites B, N, and O. Site O was mown unexpectedly early in 2001 (Table 1) causing the loss of two transition matrices of *C. jacea*, as all flowering stalks were removed. The other two species flowered before (*H. radicata*) or after (*S. pratensis*) mowing in 2001 in site O, and they were censused as in the other years. Permanent plots (five, eight, three, six, and four in sites B, L, V, N, and O respectively) of 1x1m² were established in April 1999. A border zone of 15 cm around these plots was left undisturbed. All rosettes of the studied species in a plot were mapped with 5 mm

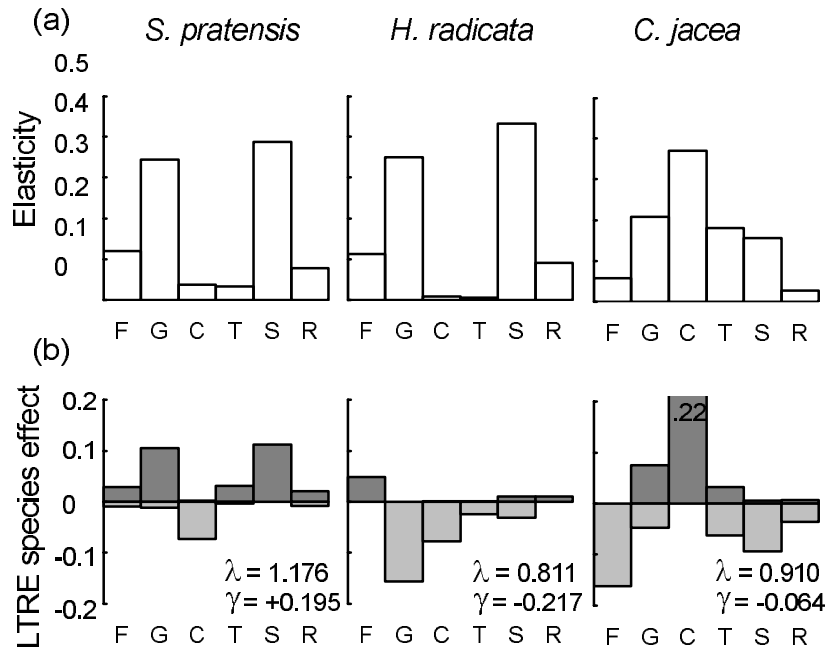


Figure 2.

(a): Mean elasticity per species (*Succisa pratensis*, *Hypochaeris radicata* and *Centaurea jacea*) grouped by six life history components: fecundity (F), growth (G), ramification (C), survival of side rosettes formed in the previous year (T), stasis (S), and retrogression (R).
 (b): Positive and negative species effect (γ^q) grouped by the life history components in the three-way LTRE analysis of the variation in population growth rate between the matrices of the three species in sites N and O over four years.

accuracy, and their establishment, survival, growth, and flowering were studied once every year during flowering (see Table 1 for exact census dates). For *S. pratensis* we randomly omitted six and three plots in sites L and N respectively, because enough (>250) rosettes were measured in the remaining plots. Plant size was quantified as the number and maximum length of rosette leaves, and the number of flowering stems and stem leaves. Finally the number of flower heads was counted and their phenological stage at the moment of census recorded: bud, flowering, seeded, or disseminated.

Stage and size classes

Classification of individual rosettes was based on a combination of size, flowering status and the formation of side-rosettes. We distinguished six classes:

- 1 Seedlings (*sdI*) are tiny rosettes with no leaves longer than 2 cm (*S. pratensis* and *H. radicata*) or 5 cm (*C. jacea*). Note that we use 'seedling' in a broad sense, and that may actually also be older very small rosettes.
- 2 Small vegetative rosettes (*sml*) are larger than seedlings but with no leaves longer than 5 cm (*S. pratensis* and *H. radicata*) or 12.5 cm (*C. jacea*).
- 3 Large vegetative rosettes (*lrg*) have longer leaves than small rosettes.
- 4 Flowering rosettes (*flow*) have at least one flowering stem and are grouped irrespective of rosette size.
- 5 Vegetative side-rosettes (*side.veg*) are newly formed by ramification and have no flowering stems.
- 6 Flowering side-rosettes (*side.flow*) are newly formed by ramification and have at least one flowering stem.

The first four classes largely correspond to an earlier classification for *S. pratensis* (Bühler & Schmid 2001). No seed stage is modelled as these species are known to have predominantly transient seed banks (Thompson et al. 1997).

Ramification resulted in new rosettes close to old rosettes. It is not always possible to determine from which old rosette a new clonal rosette originates without disturbance. Therefore we assigned all new arisen, non-seedling rosettes to the nearest old, non-seedling rosette, when they were not further than a species-specific distance apart. Based on field experience of the species, we arbitrarily set this distance to 25, 10 and 40 mm for respectively *S. pratensis*, *H. radicata* and *C. jacea*. In those cases where it was possible to determine connections without disturbance ($n=63$ for *S. pratensis*, $n=18$ for *H. radicata* and $n=200$ for *C. jacea*), more than 92% of the distances between old and new rosettes were not larger than the species-specific distance for each species. In *C. jacea* side-rosettes were only appointed to non-flowering rosettes when no flowering rosettes had been present within the 40 mm radius, because 89% of the 200 known ramified rosettes were attached to flowerings stems.

Matrix parameterization

With the calculated year-to-year transition probabilities we constructed 6x6 projection matrices of the form A in which each element (a_{ij}) represents the transition from the j^{th} category in year t to the i^{th} category in year $t+1$. The 36 matrix elements were classified into the following life history components: fecundity (F), growth (G), ramification (C), survival of side-rosettes that were formed in the previous year (T), stasis (S), and retrogression (R) (Table 2). Growth is defined as the transition from a smaller and vegetative stage class to a larger or flowering stage class by a surviving rosette. Stasis means that a rosette remains in the same class, and rosettes that retrogress survive but get smaller in the hierarchy of classes.

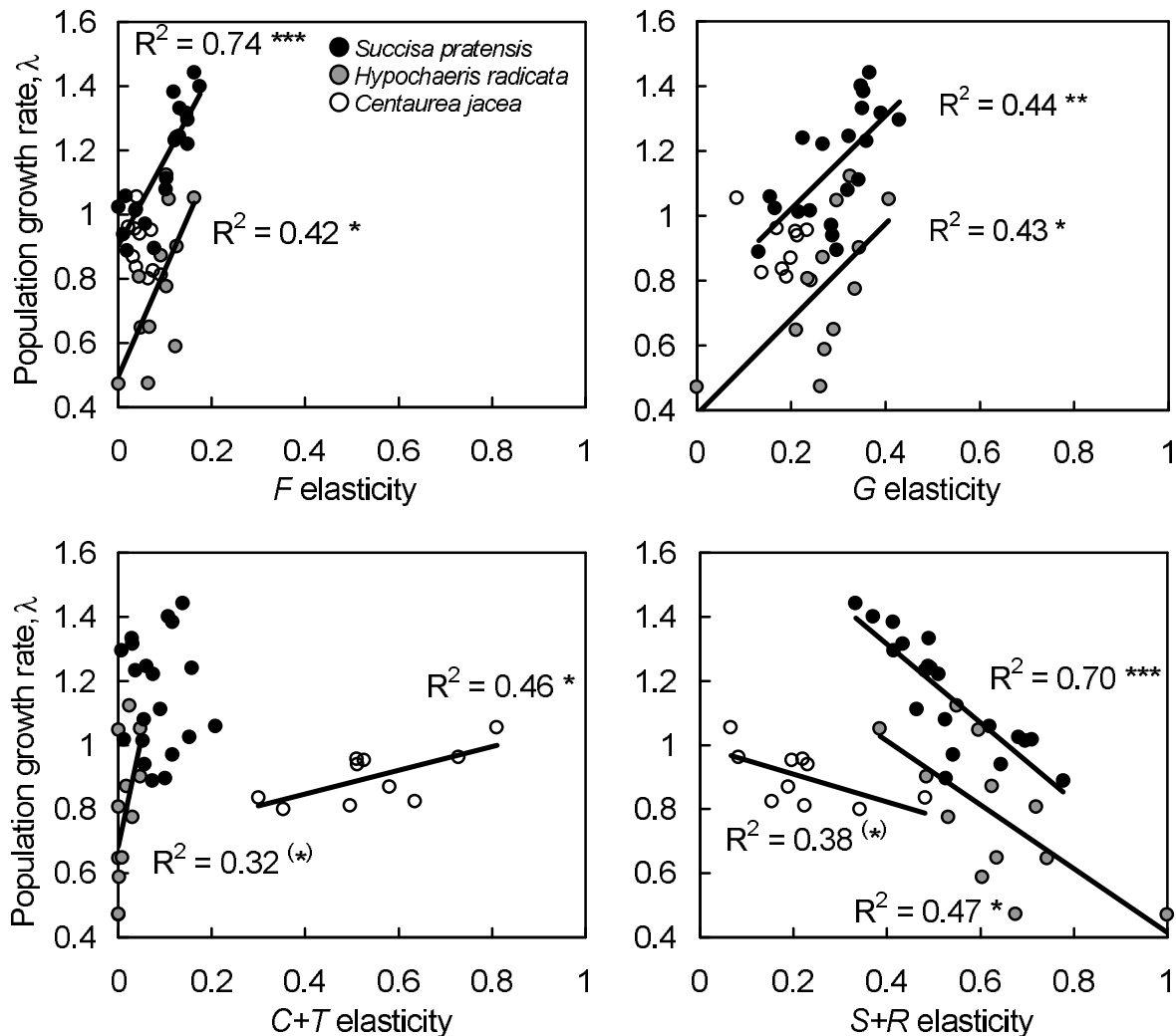


Figure 3.

Correlation between projected population growth rate (λ) and the summed elasticity of different life cycle components: fecundity (F), growth (G), clonal propagation and survival of side rosettes formed in the previous year ($C+T$), and survival and retrogression ($S+R$). Please note that the elasticities of a matrix sum to one, and that the x-axes of the four diagrams are therefore negatively correlated. Each dot represents an individual transition matrix. Significant, linear regression lines are shown for each species. (*) = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

In the first year (1999 to 2000) it was not possible to observe fates of side-rosettes formed in the previous year, because no observations were made in the 1998, resulting in empty classes of new clonally-produced rosettes that were formed in 1998. For those first year classes (*side.veg* and *side.flow*) and for stage classes containing less than six rosettes at time t , the transition probabilities of that class to all other classes at time $t+1$ was estimated from all observations over the years on that class in the same population. The numbers of observations was only in 9% of the instances smaller than six in the adult classes *sml*, *lrg* and *flow*, but more often in the clonal offspring classes as ramification was a rare event in *H. radicata* and *S. pratensis*.

Seedling establishment and fate were studied in a seed addition experiment since the density of seedlings was much lower than that of adult plants. In November 1999 and 2000, seeds were added to 5 by 50 cm rectangular plots in sites B, N, and O. One hundred seeds were sown in each of 10 plots per year and per site. The number of seedlings and small rosettes just before and 12 and 24 months after seed addition was compared to control plots. Seedling and small rosette establishment per seed (p_{sdl} and p_{sml} respectively) were calculated as follows:

$$p_{sdl} = \frac{(N_{sdl,t=1}^{SA} - N_{sdl,t=0}^{SA}) - (N_{sdl,t=1}^{NS} - N_{sdl,t=0}^{NS})}{N_{seeds}^{SA}} \quad (1)$$

$$p_{sml} = \frac{(N_{sml,t=1}^{SA} - N_{sml,t=0}^{SA}) - (N_{sml,t=1}^{NS} - N_{sml,t=0}^{NS})}{N_{seeds}^{SA}} \quad (2)$$

where N is the number of seeds, seedlings (*sdl*) or small rosettes (*sml*) in either the seed addition plots (SA) or control plots (NS) at the beginning ($t=0$) of the experiment or one year later ($t=1$). These per seed establishment probabilities, p , were multiplied by the average seed production of the average flowering rosette per population, in order to get the sexual reproduction matrix elements, F_{ij} . Per rosette seed production was estimated as the product of the mean number of observed flower heads and the population average number of seeds per top flower head (Table 3). In *S. pratensis* only flower heads that flowered or had finished flowering when censused were considered in this calculation, because we assumed that the remaining buds of flower heads would be lost by mowing in this late flowering species. In the other two species flower head buds were counted as well, since these earlier flowering species were monitored earlier on average.

Seedling fates, S_{11} and G_{21} , were derived from the same seed addition experiment because very low numbers of seedlings were observed in the permanent plots. Seedling stasis, S_{11} , was calculated as follows:

$$S_{11} = \frac{N_{sdl,t=2}^{SA}}{N_{sdl,t=1}^{SA}} \quad (3)$$

in which the plots per site were pooled. Seedling growth, G_{21} , was calculated as the relative increase in the number of small rosettes in the seeds added plots from 1 to 2 years after seed addition for each seedling present after one year. The annual

mortality rate of small rosettes was also accounted for and estimated from the permanent plot data. Seedling growth, G_{21} , was computed as:

$$G_{21} = \frac{N_{sml,t=2}^{SA} - N_{sml,t=1}^{SA} \bullet (R_{12} + S_{22} + G_{32} + G_{42})}{N_{sdl,t=1}^{SA}} \quad (4)$$

in which the plots were pooled for each site and starting year, and the sum of R_{12} , S_{22} , G_{32} and G_{42} was the average survival probability of small rosettes.

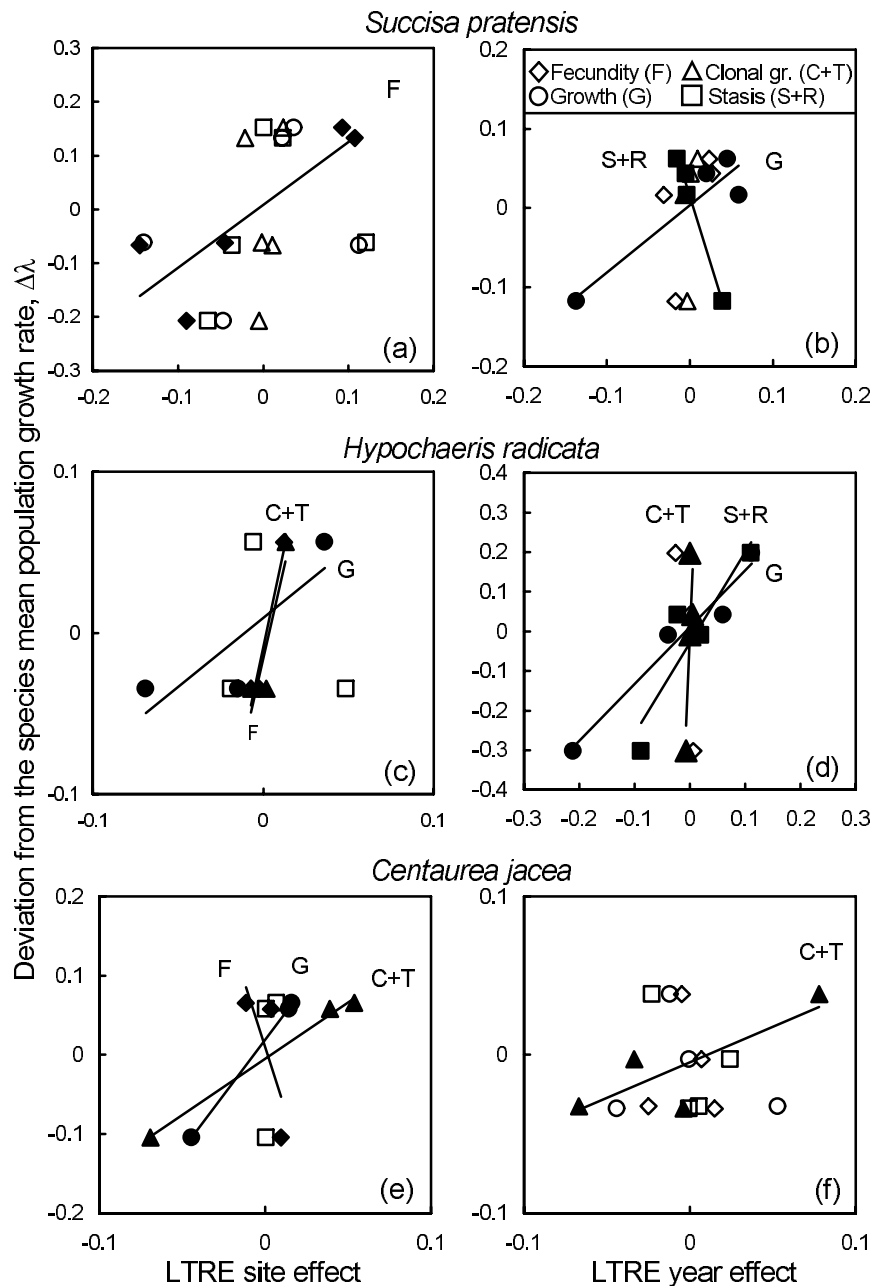


Figure 4.

Relationships between the deviation from the species mean population growth rate, $\Delta\lambda$, and the main effects of site and year in the variation decomposition (LTRE) in different species, and per life history component: fecundity (F), growth (G), ramification and survival of side rosettes formed in the previous year (C+T), and stasis and retrogression (S+R). Black symbols and trend lines signify regressions with $R^2 > 0.50$. Please note that the axes of different diagrams can have different scales.

Matrix analysis

The Matlab student edition of 1996 was used for all matrix computations. For each population transition matrix we calculated the projected population growth rate, λ , which is the dominant eigenvalue of a matrix. The elasticity of λ for each matrix element (e_{ij}) (de Kroon et al. 1986) is given by:

$$e_{ij} = \frac{a_{ij}}{\lambda} \frac{\partial \lambda}{\partial a_{ij}} \quad (5)$$

Elasticity values quantify the relative contribution of each element to λ , and sum to one. We added the elasticity values of all transitions of the same life cycle component for each matrix separately. The transition from a flowering stage to small vegetative rosettes is the sum of a fecundity and a retrogression component. As the elasticity of these matrix elements could not be divided into these two components, it was completely assigned to fecundity, which forms the major part of these matrix elements (82% in *S. pratensis*, 80% in *H. radicata* and 100% in *C. jacea*). To construct 95% confidence intervals for the projected population growth rates (λ), we applied the bootstrapping method by resampling 3,000 times the data set of observed rosette fates with which a particular matrix was constructed (Efron 1982; Kalisz & McPeck 1992; Caswell 2001). The survival (T , G , S , and R) and ramification (C) observations were resampled for all classes separately. New sexual reproduction (F) elements were calculated after resampling the accompanying data set of number of flower heads of the observed flowering rosettes. The upper and lower limits of the 95% confidence intervals were adjusted for small deviations between the mean λ of the 3,000 newly constructed matrices and the λ of the original matrix (Caswell 2001, page 306).

To decompose variation in λ we applied factorial life table response experiments (LTRE). The LTRE model with two factors within a species is (Caswell 2001):

$$\lambda^{(mn)} \equiv \lambda^{(\cdot\cdot)} + \alpha^m + \beta^n + (\alpha\beta)^{(mn)} \quad (6)$$

in which a given λ of the m^{th} site and n^{th} year is written as the sum of the dominant eigenvalue of the mean of all matrices of a species, $\lambda^{(\cdot\cdot)}$, the main effect of the m^{th} site, α^m , the main effect of the n^{th} year, β^n , and the residual 'interaction' effect, $\alpha\beta^{(mn)}$ (Horvitz et al. 1997). The main effects can be estimated by filling in this equation first for each level of the main effects separately, while ignoring the interaction term. The main and interaction effects can then be decomposed into contributions from each matrix element (Caswell 2001):

$$\tilde{\alpha}^m = \sum_{i,j} (a_{ij}^{(m\cdot)} - a_{ij}^{(\cdot\cdot)}) \frac{\partial \lambda}{\partial a_{ij}} \bigg|_{\frac{1}{2}(A^{(m\cdot)} + A^{(\cdot\cdot)})} \quad (7)$$

$$\tilde{\beta}^{(n)} = \sum_{i,j} (a_{ij}^{(n)} - a_{ij}^{(\cdot)}) \frac{\partial \lambda}{\partial a_{ij}} \bigg|_{\frac{1}{2}(A^{(n)} + A^{(\cdot)})} \quad (8)$$

$$\left(\tilde{\alpha} \tilde{\beta} \right)^{(mn)} = \sum_{i,j} (a_{ij}^{(mn)} - a_{ij}^{(\cdot)}) \frac{\partial \lambda}{\partial a_{ij}} \bigg|_{\frac{1}{2}(A^{(mn)} + A^{(\cdot)})} - \tilde{\alpha}^m - \tilde{\beta}^n \quad (9)$$

in which differences of each matrix element with the corresponding matrix element of the overall mean matrix, $A^{(\cdot)}$, are multiplied by the sensitivity values of the matrix halfway the matrix of interest and the overall mean matrix. The resulting contribution matrices of the main and interaction effects may contain both positive and negative values. The magnitude of the sum of all elements of a contribution matrix is a rough summary measure of the effect of a factor on λ (Horvitz et al.1997).

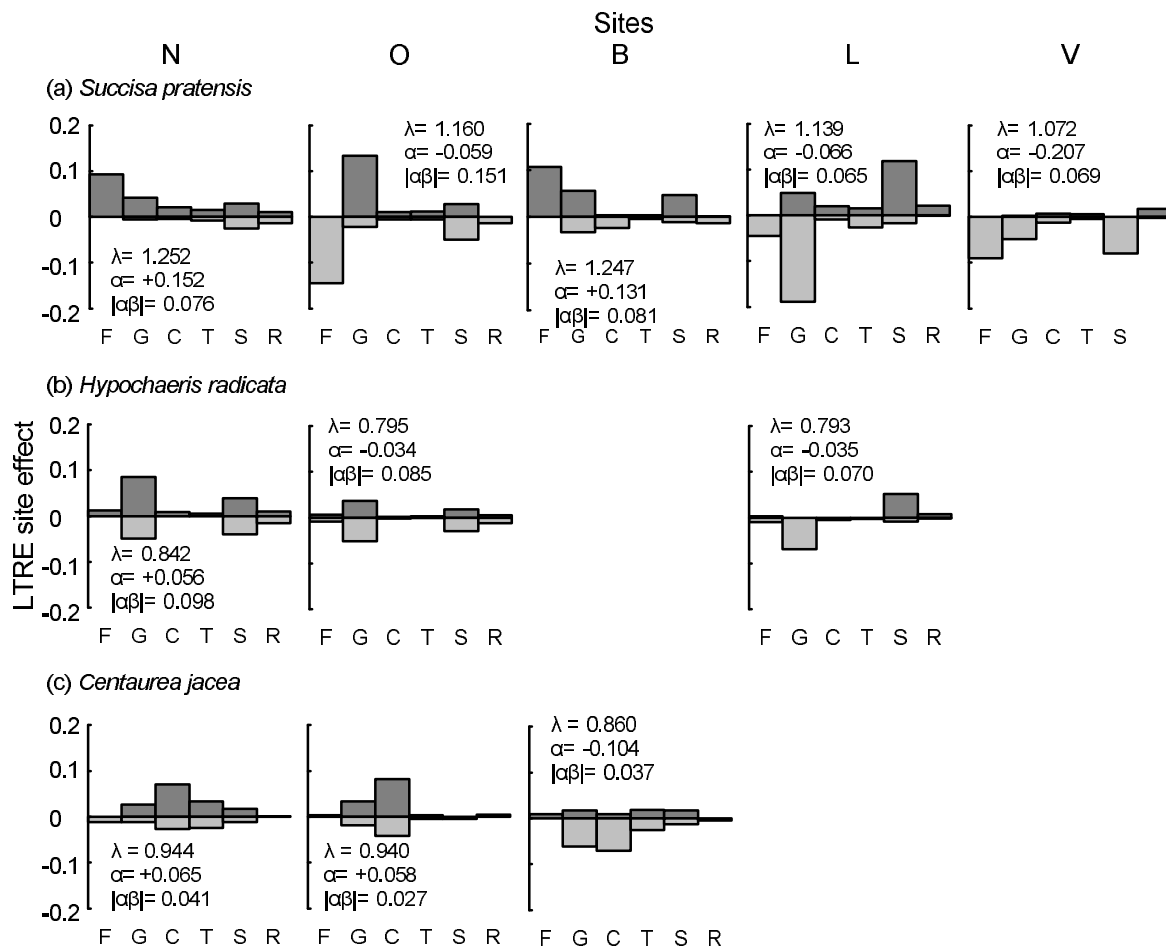


Figure 5.

Site effects in the decomposition of variation (LTRE) in population growth rate (λ) in *Succisa pratensis* (top row), *Hypochaeris radicata* (middle row) and *Centaurea jacea* (bottom row). Contributions of positive and negative matrix elements are separately grouped by life history component: fecundity (F), growth (G), ramification (C), survival of side rosettes formed in the previous year (T), stasis (S) and retrogression (R). For each site m and species the average site mean λ , site effect (α^m), and the mean absolute value of the interaction effect $\alpha\beta^{(mn)}$ is given. The overall species mean λ is 1.176, 0.811 and 0.910 respectively.

However, information on the magnitude and direction (positive or negative) of the elements of the contribution matrix is lost in this measure. Therefore we summed the values of these elements for each of the six life history components (F , G , C , T , S , and R) for each of the transition matrices. Positive correlations between LTRE contributions and deviations from the mean λ are expected for those components whose variation contributes consistently to the variation in λ among the years or sites.

If trade-offs between life history components exist, a combination of negative and positive contributions within a matrix can be expected in a variance decomposition analysis (cf. Sterck et al. 2003). Therefore we separated the positive and negative elements within life history components in order to see whether and how positive contributions by some elements were compensated for by negative contributions of others. Note that if a given matrix has a λ smaller than the λ of the overall mean matrix the sum of all LTRE contributions will be negative, but positive LTRE contributions of some elements may buffer the negative contributions of the other elements partly. Similar buffering occurs by elements with negative contributions for matrices with high lambdas. Sterck et al. (2003) showed that life history trade-offs may thus dampen the variation in population growth rate.

To investigate the relative performance of the three coexisting species together, we also performed a three-way LTRE on the matrices constructed for the N and O populations of all three species. The reference matrix, $A^{(+)}$, in this case was calculated as the mean of the three species mean matrices.

Results

Life cycle and elasticity analyses

The projected population growth rates (λ) were higher in *Succisa pratensis* than in the other two species: 55% of the lambdas were significantly larger than unity in *S. pratensis*, vs. 9% in the other species (Fig. 1). Whereas the lambdas of the *Centaurea jacea* populations did not differ much between years, there was a tendency to a decrease in λ through time in *S. pratensis* and especially in *Hypochaeris radicata*.

Elasticity analysis showed the highest summed elasticities for growth (G) and stasis (S) in *S. pratensis* and *H. radicata* and for ramification (C) in *C. jacea* (Fig. 2). There were significantly positive correlations between λ and the summed elasticities for fecundity (F) and growth (G) in *S. pratensis* and *H. radicata* whereas clonal life history components ($C+T$) were positively correlated with λ in *C. jacea* (Fig. 3). Because the elasticities of each matrix sum to one, there were also negative correlations. Projection matrices with lower lambdas had higher elasticities for stasis and retrogression ($S+R$), but most strongly so in *S. pratensis* and *H. radicata*. Remarkably, the long-lived *S. pratensis* and the short-lived perennial *H. radicata* had comparable elasticity matrices as the elasticity values of the different components

have similar ranges in both species and because the regression lines are parallel.

Life Table Response Experiment analyses

The first-order approximation model of LTREs within species fitted well. The difference between the observed and modelled lambdas were less than 1% on average, though in six out of 42 cases the difference was larger than 1%, with a maximum of 6%. The three-way LTRE also fitted well with an average difference of 1.6%.

The three-way (species, site and year) decomposition of the variation in λ of populations in sites N and O revealed that species was the strongest main factor. After the intermediate effects of year, the smallest contribution to the variation in λ was

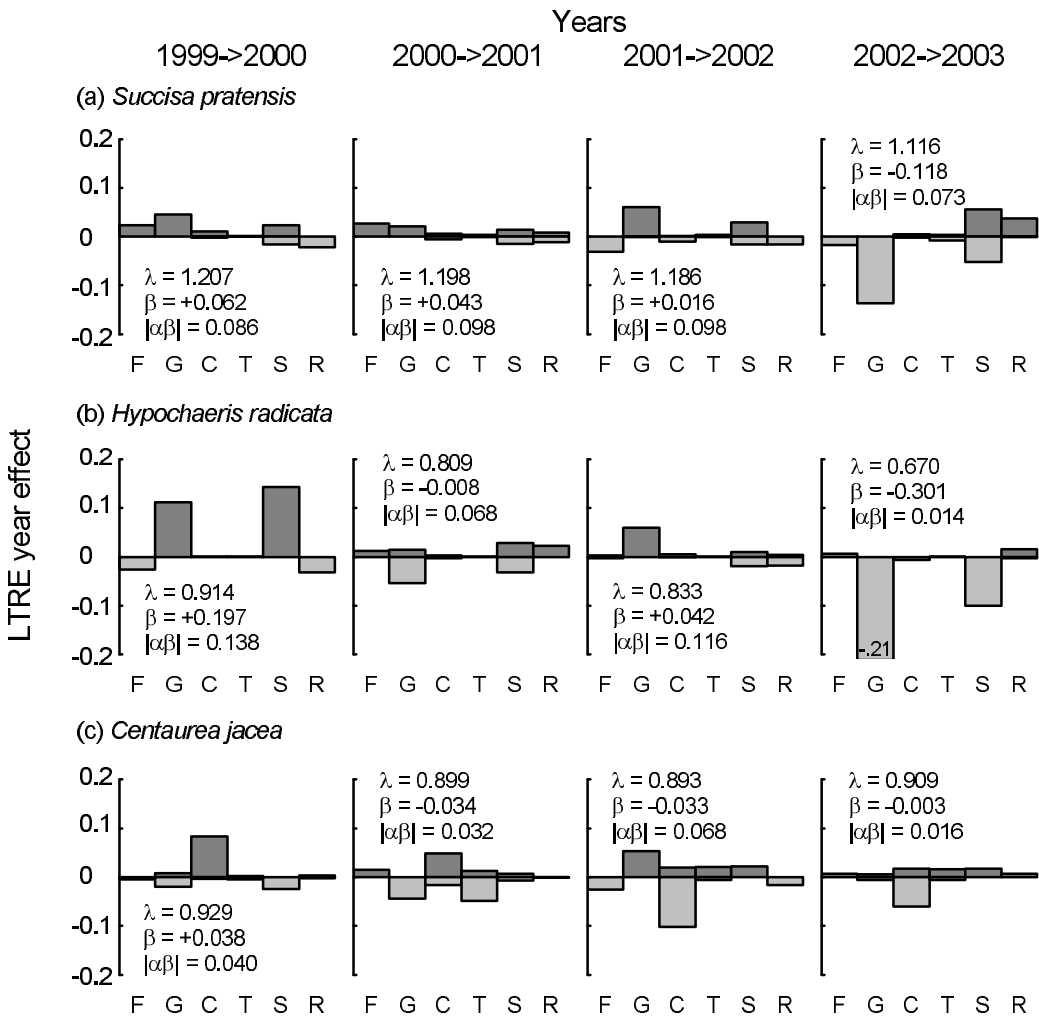


Figure 6.

Year effects in the decomposition of variation (LTRE) in population growth rate (λ) in *Succisa pratensis*, *Hypochaeris radicata* and *Centaurea jacea*. Contributions of positive and negative matrix elements are separately grouped by life history component: fecundity (F), growth (G), ramification (C), survival of side rosettes formed in the previous year (T), stasis (S) and retrogression (R). For each year n and species the average site mean λ , year effect (β^n), and the mean absolute value of the interaction effect $\alpha\beta^{(mn)}$ is given. The overall species mean λ is 1.176, 0.811 and 0.910 respectively.

Table 1.

Census and mowing dates for each year for each population of *Succisa pratensis*, *Hypochaeris radicata* and *Centaurea jacea*. The sites are Konijnendijk (N), Koolmansdijk (O), Bennekomse meent (O), Leemputten (L) and Veerslootlanden (V).

Site	Year	<i>S. pratensis</i>	<i>H. radicata</i>	<i>C. jacea</i>	Mown
N	1999	21-Sep	7-Jun	21-Sep	18-Oct
N	2000	13-Sep	7-Jun	6-Jul	13-Sep
N	2001	17-Sep	22-Jun	6-Sep	15-Oct
N	2002	2-Sep	21-Aug	2-Sep	5-Sep
N	2003	4-Sep	4-Jul	18-Aug	10-Sep
O	1999	9-Jun	9-Jun	9-Jun	22-Aug
O	2000	16-Aug	6-Jun	6-Jul	25-Aug
O	2001	2-Oct	12-Jul	-	23-Jul
O	2002	23-Aug	2-Aug	2-Aug	9-Oct
O	2003	23-Jul	23-Jul	23-Jul	1-Aug & 2-Oct
B	1999	20-Jul	-	20-Jul	15-Aug
B	2000	26-Jul	-	26-Jul	5-Aug
B	2001	31-Jul	-	31-Jul	5-Aug
B	2002	26-Jul	-	26-Jul	22-Aug
B	2003	22-Jul	-	22-Jul	12-Aug
L	1999	23-Sep	23-Sep	-	15-Oct
L	2000	6-Oct	9-Jun	-	15-Nov
L	2001	3-Oct	11-Jul	-	-
L	2002	3-Oct	1-Aug	-	-
L	2003	5-Sep	23-Jul	-	-
V	1999	7-Sep	-	-	15-Sep
V	2000	7-Sep	-	-	15-Sep
V	2001	5-Sep	-	-	15-Sep
V	2002	4-Sep	-	-	15-Sep
V	2003	5-Sep	-	-	15-Jun

made by the factor site (Table 4). The effects of the interactions between the main factors, however, were as large as the main site and almost as large as the main year effect. After subtracting the LTRE contributions of the main effects of species and sites, *S. pratensis* performed better than average in site N ($\alpha\gamma = +0.046$) while *C. jacea* performed less well in that site ($\alpha\gamma = -0.038$). The opposite pattern was found in site O ($\alpha\gamma = -0.077$ for *S. pratensis* and $\alpha\gamma = +0.037$ for *C. jacea*). *Hypochaeris radicata* deviated little from the species and site averages ($\alpha\gamma = +0.002$ for site N and for site O $\alpha\gamma = -0.004$). However, when the main species and year contribution matrices were subtracted *S. pratensis* showed the smallest deviation from the averages (mean $|\beta\gamma| = 0.015$ vs. 0.081 for *C. jacea* and 0.055 for *H. radicata*) indicating that *S. pratensis* followed the mean year effect most closely. Especially in the last year *C. jacea* performed better ($\beta\gamma = +0.128$) and *H. radicata* worse ($\beta\gamma = -0.121$) than average.

Within-species variation in λ decomposed differently into site, year and interaction effects in the different species. In *S. pratensis* the main site effects were stronger than year and interaction effects (Table 4). Over all sites contributions of fecundity (F) were positively correlated with site effects (α^m) (Fig. 4a). Analysing the components that explained the deviation in λ for each of the sites separately, it was apparent that especially the F , G and S components displayed high positive

contributions in sites with higher $\bar{\epsilon}$ than average ($\alpha^N = +0.152$ and $\alpha^B = +0.131$; Fig. 5a), and almost all their matrix elements were higher than average. The reverse was true for site V. For this site, the very low λ ($\alpha^V = -0.207$) was due to an overwhelming effect of negative contributions (-0.239) with little positive contributions (+0.032). In contrast to site V, the other two bad sites had an average λ only slightly lower than the overall mean λ ($\alpha^O = -0.059$ and $\alpha^L = -0.066$) because the large negative contributions (-0.267 on average) were compensated to a large extent by positive contributions (+0.204). In site O low values for F elements were partly compensated for by high values for G elements; in site L negative G contributions were buffered by a high S contributions.

The year effects (mean $|\beta| = 0.060$) in *S. pratensis* were not only smaller than the site effects (mean $|\alpha| = 0.123$), but also qualitatively different. In contrast to the differences between sites (mean $|\alpha| = 0.096$ for F , Fig. 5a), F did not contribute much to variation in λ between years (mean $|\beta| = 0.025$ for F ; Fig. 6a). Growth (G) contributions, however, covaried positively with λ variation over years (Fig. 4b), but did not correlate with λ variation over sites. Especially in the last year, which had the lowest λ , G and S had large negative contributions. This indicates that rosettes grew less often to larger classes in this year. Because they stayed small, or even retrogressed, some S and R element were actually higher than average resulting in positive LTRE contributions that buffered the predominantly negative contributions.

In the shorter-lived *H. radicata*, site effects were smaller than year effects (Table 4). The contributions to λ by life history components were more consistent between site and year effects than they were in *S. pratensis*. Growth (G) covaried strongly with λ in both cases and other components had less effect (Fig. 4c,d). In all three sites positive and negative contributions of G and S over the years balanced in the populations of *H. radicata* (Fig. 5b). The largest contributions to the variation in λ between years were made by G and S , which were both strongly positive in the first year and strongly negative in the last year (Fig. 6b).

Centaurea jacea showed little variation in λ (Fig. 1), and therefore the different effects were also smaller with site effects being the most important (Table 4). Clonal propagation (C) contributions had the strongest correlations with variation in λ , both between sites and between years (Fig. 4e,f). Variation in F was negatively correlated with variation in λ between sites, but not between years. Within sites clonal propagation (C), and in site B also growth (G), made the largest contributions. But whereas contributions of all life history components were predominantly either positive or negative in a given site (Fig. 5c), this was less the case in especially the bad years. In such years positive and negative contributions occurred simultaneously (Fig. 6c).

Table 2.

Population matrix divided into life history components and mean matrices per species. The six stage classes are: very small vegetative rosettes or seedlings (*sdl*), small vegetative rosettes (*sml*), large vegetative rosettes (*lrg*), rosettes with at least one flowering stem (*flow*), vegetative rosettes produced by ramification (*side.veg*), rosettes produced by ramification with at least one flowering stem (*side.flow*). The six life history components are fecundity (F_{ij}), growth (G_{ij}), ramification (C_{ij}), survival of side rosettes formed in the previous year (T_{ij}), stasis (S_{ij}) and retrogression (R_{ij}). Matrix elements are means of 20 transition matrices of *Succisa pratensis*, 12 of *Hypochaeris radicata* and 10 of *Centaurea jacea*. Beside each mean the coefficient of variation (CV) is given.

	sdl		sml		lrg		flow		side.veg		side.flow	
sdl	S_{11}		R_{12}		-		F_{14}		-		F_{16}	
sml	G_{21}		S_{22}		R_{23}		$F_{24}(+R_{24})$		T_{25}		$F_{26}(+R_{26})$	
lrg	-		G_{32}		S_{33}		R_{34}		T_{35}		T_{36}	
flow	-		G_{42}		G_{43}		S_{44}		T_{45}		T_{46}	
side.veg	-		C_{52}		C_{53}		C_{54}		C_{55}		C_{56}	
side.flow	-		C_{62}		C_{63}		C_{64}		C_{65}		C_{66}	
<i>S. pratensis</i>	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
sdl	0.489	0	0.033	154	-		0.994	90	-		0.994	90
sml	0.370	0	0.306	69	0.075	87	0.677	84	0.295	84	0.750	68
lrg	-		0.416	56	0.566	25	0.413	50	0.451	45	0.680	40
flow	-		0.060	156	0.280	52	0.471	42	0.155	90	0.180	127
side.veg	-		0.041	112	0.038	104	0.105	89	0.033	184	0.200	126
side.flow	-		0.002	365	0.002	222	0.013	112	0.000		0.000	
<i>H. radicata</i>	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
sdl	0.375	0	0.033	126	-		1.743	30	-		1.743	30
sml	0.163	0	0.260	63	0.111	78	0.572	26	0.184	75	0.334	30
lrg	-		0.282	76	0.479	38	0.349	30	0.107	94	0.111	148
flow	-		0.033	147	0.135	95	0.190	71	0.000		0.111	148
side.veg	-		0.015	231	0.011	105	0.025	118	0.000		0.000	
side.flow	-		0.000		0.005	193	0.007	300	0.000		0.000	
<i>C. jacea</i>	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
sdl	0.498	0	0.026	131	-		0.199	52	-		0.199	52
sml	0.399	0	0.199	66	0.148	37	0.050	52	0.222	68	0.050	52
lrg	-		0.240	63	0.318	34	-		0.276	61	-	
flow	-		0.109	93	0.266	58	-		0.268	33	-	
side.veg	-		0.109	126	0.081	74	0.390	42	0.098	59	0.522	43
side.flow	-		0.035	170	0.012	155	0.591	54	0.016	84	0.272	27

Discussion

Life cycle and elasticity analyses

The average elasticity patterns suggest that *Succisa pratensis* and *Hypochaeris radicata* have comparable life-cycles. This is probably due to the fact that the population growth rates (λ) of the long-lived perennial (*S. pratensis*) were higher than those of the short-lived species (*H. radicata*). When matrices with the same λ (e.g. $\lambda = 1$) are compared *S. pratensis* relies more strongly on rosette survival as would be

expected of a longer-lived species (Silvertown et al. 1993). This stresses that elasticities are properties of a particular matrix and that caution is needed when comparing elasticities of different matrices within and between species (Shea et al. 1994; de Kroon et al. 2000; Caswell 2001). Within our three species λ was positively correlated with the elasticities of fecundity (F), G and C and negatively with the elasticities of retrogression (R) and S , which is consistent with patterns in other species (Silvertown et al. 1993; Oostermeijer et al. 1996; de Kroon et al. 2000).

The ramification rates of the meadow populations of *H. radicata* were much lower than those of road-verges (de Kroon et al. 1987; van Groenendael et al. 1994). The importance of clonality in *C. jacea* is related to the monocarpic nature of the rosettes that die after flowering. In this species clonal propagation, the sprouting of new rosettes at the base of the old stem, is the only way to survive for a flowering individual (Hartemink et al. 2004). In these meadows the number of connected rosettes rarely exceeds three, whereas *C. jacea* can form extensive below-ground woody branches in more productive habitats as dykes and road verges (E. Jongejans, personal observation).

Life table response experiments

The three-way life table response experiment (LTRE) showed that the variation between the species was most important for the observed variation in λ , followed by year variation and then by site variation. This suggests that co-occurring species, given their specific life history, are relatively more synchronized in time than that they are similar in space. The synchrony occurred because one year (1999 to 2000) had the highest growth rates in all species, while the last year (2002 to 2003) was always below average, probably because of the dry early spring and summer of 2003. But the magnitude of the temporal variation differed between species: environmental year-to-year fluctuations are better buffered by the clonal life history of the woody *C. jacea*, than by the long-lived *S. pratensis*, and even less so by the shorter-lived *H. radicata*. In *S. pratensis* LTRE and elasticity analyses point at the same major players: growth (G), stasis (S) and fecundity (F). This indicates that those life history components that contribute most to local population growth (λ), also contribute most, by their variation, to the spatiotemporal variation in λ . A resemblance of the outcome of these different types of analyses was also found by Horvitz et al. (1997), Eriksson & Eriksson (2000), Kiviniemi (2002), Nordbakken et al. (2004) and Endels (2004).

Temporal and spatial variation in population dynamics

Contributions of variation in life history components to variation in λ were both qualitatively (direction) and quantitatively (magnitude) different for site effects and year effects. This suggests that year and site variation influence population dynamics in very different ways. Like in the perennial herb *Lathyrus vernus* (Ehrlén 1995) site

Table 3.

Mean number of seeds per flower head and mean number of flower heads per flowering rosette of *Succisa pratensis*, *Hypochaeris radicata* and *Centaurea jacea*. Seeds were counted in randomly selected top flower heads per population (data from all sampled years are pooled). The number of flower heads per flowering rosette were derived from permanent plots for each site and year combination separately. The sites are Konijnendijk (N), Koolmansdijk (O), Bennekomse meent (O), Leemputten (L) and Veerslootlanden (V).

Species	Site	Flower heads sampled				Permanent plots			
		Years	n	No. Seeds		No. flower heads per flowering rosette			
				mean	s.e.	1999	2000	2001	2002
<i>S. pratensis</i>	N	'01,'02	49	48	1.7	4.44	3.00	0.87	1.56
<i>S. pratensis</i>	O	'00,'01,'02	54	39	2.9	0.00	0.00	1.56	0.19
<i>S. pratensis</i>	B	'99,'00,'02	70	63	1.6	2.36	2.57	1.92	2.35
<i>S. pratensis</i>	L	'99,'00,'01	80	30	1.6	0.70	2.04	1.57	1.29
<i>S. pratensis</i>	V	'99,'00,'02	73	46	1.9	0.39	0.89	0.44	0.64
<i>H. radicata</i>	N	'00,'01,'02	53	53	3.2	1.00	2.85	2.44	2.22
<i>H. radicata</i>	O	'99,'00,'01,'02	75	45	2.1	3.11	2.63	2.31	2.73
<i>H. radicata</i>	L	'01	22	34	4.5	0.90	2.60	3.00	3.00
<i>C. jacea</i>	N	'00,'01,'02	75	23	1.7	1.51	1.53	0.35	1.08
<i>C. jacea</i>	O	'99,'00,'02	69	36	2.3	0.25	3.30	-	1.69
<i>C. jacea</i>	B	'99,'00,'02	70	29	2.4	1.61	1.85	1.10	0.91

effects were also stronger than year effects in *S. pratensis*. Different sites and years differed especially in the way low growth rates of the *S. pratensis* populations were realized. While the differences between temporal and spatial effects were much smaller in *H. radicata* and *C. jacea*, also in these species fecundity covaried with variation in λ between sites and not between years. High fecundity has frequently been reported to cause high λ , e.g. in the non-clonal *Centaurea corymbosa* (Fréville et al. 2004), but no distinction was made between spatial and temporal variation in that study.

Large interaction effects between sites and years would mean that demographic variation over time is weakly correlated among populations (Menges 2000). In our case, however, the interaction effects were always intermediate in strength between the site and the year effect. *Hypochaeris radicata* showed marked population declines in all three sites over time. The last year, which was also the worst year for *S. pratensis*, may partly be explained by the above-mentioned drought. Another explanation may be found in the higher spatial dynamics of short-lived species. By selecting spots for permanent plots in which *H. radicata* rosettes were present at the beginning of our study we may have been biased to find population decline (Crawley 1990), but as we did not observe high colonization rates in the vicinity of the plots, this is unlikely to be the main explanation for the observed population decline.

Flexibility in life history responses

In two of the three sites in which *S. pratensis* performed below average, large negative LTRE contributions were almost completely buffered by positive contributions of other life history components, resulting in a small, negative net effect. Buffering of negative by positive contributions of different elements of the life cycle seems to be a more general pattern as negative covariances between life history components were also reported by Picó et al. (2002) and Sterck et al. (2003). These negative correlations may reflect trade-offs between life history functions. For instance, the most nutrient-poor site L, in which *S. pratensis* plants stayed small, showed negative contributions of growth (G) and compensating positive contributions of stasis (S) when compared to the other sites. This can be understood by the observation that rosette growth (G) was reduced but not survival ($G+S+R$). Interestingly *H. radicata* showed the same pattern in site L. Similar trade-offs between different fates of surviving meristems were found in the tropical tree *Vouacapoua americana* (Sterck et al. 2003).

In the second bad site (O) a negative LTRE contribution of F (caused by mowing before seed set) was partly compensated for by a positive G contribution. A demographic trade-off between F and G may be expected because Hartemink et al. (2004) experimentally found costs of seed production in *S. pratensis* expressed as reduced vegetative growth. However, such a trade-off is unlikely to fully explain the high G contribution as G may also be high due to favourable growing conditions in this somewhat drier site. Here, external factors may underlie the specific LTRE patterns, and experimentation would be required to compare their importance compared to those of trade-offs. In the third bad site (V) all vital rates were consistently lower or unchanged. Here a dense grass vegetation probably consistently reduced S , G and F .

In *C. jacea* indications of compensation by different life cycle components were only found in bad years. LTRE contributions of clonal propagation (C) seem to be buffered by opposing contributions of especially growth (G) and survival of clonal offspring (T). This would suggest that climatic variation influences plant developmental decisions rather than overall growing conditions for this species as \bar{e} varies little. Picó et al. (2002) also pointed at temporal variation in climate (precipitation) as the likely explanation for the negative covariances of seedling production in different periods of the flowering season of the perennial herb *Lobularia maritima*.

Buffering of negative contributions to variation in λ , irrespective the underlying mechanisms, is important for the population dynamics as it reduces the variance in population growth rate. Lower variance in λ leads to lower population extinction risks (Tuljapurkar & Orzack 1980; Menges 1998). However the spatial synchrony in the dynamics of the populations of *S. pratensis* and especially *H. radicata* makes these species regionally more vulnerable to extinction (Harrison & Quinn 1989; Sutcliffe et al. 1997; Heino et al. 1997; Matter 2001).

Table 4.

Magnitude of the different effects of four variation decomposition analyses (LTRE) of variation in population growth rate, λ : all species (*Succisa pratensis*, *Hypochaeris radicata* and *Centaurea jacea*) together in site N and O over four years (three-way), and each species separately in all observed sites over four years. The mean and standard deviation (sd) of the absolute values of all levels within a LTRE effect are given (*100). The overall mean λ was 1.047, and λ was 1.176, 0.811 and 0.910 respectively for the three species separately.

LTRE effect		Three-way		<i>S. pratensis</i>		<i>H. radicata</i>		<i>C. jacea</i>	
		mean	sd	mean	sd	mean	sd	mean	sd
Site (S)	$ \alpha^m $	4.32	0.47	12.31	6.19	4.16	1.22	7.53	2.48
Year (Y)	$ \beta^n $	8.08	5.49	5.99	4.31	13.70	13.65	2.70	1.63
Species (P)	$ \gamma^q $	15.86	8.31						
S * Y	$ \alpha\beta^{mn} $	4.41	2.52	8.86	6.11	8.40	6.5	3.68	2.22
S * P	$ \alpha\gamma^{mq} $	3.38	2.80						
Y * P	$ \beta\gamma^{nq} $	5.32	4.97						
S * Y * P	$ \alpha\beta\gamma^{mnq} $	7.07	5.48						

Implications

Temporal and spatial variation in the dynamics of populations may not be readily substitutable, as we have found that good years can be different from good sites and that bad sites may also differ amongst each other. For model studies on the regional dynamics of plants this is an important point to take into consideration. The differences between temporal and spatial variation are likely to be the result of the different factors that generate the variation: year-to-year variation in meadows is mainly caused by climatic fluctuations and variation in management, whereas spatial variation has various sources including site productivity and vegetation composition.

Our LTRE analyses show that variation in very different life history components may underlie the variation in λ , even among species with similar life histories. Although *S. pratensis* and *H. radicata* showed similar responses to the spatiotemporal variation in growing conditions, the magnitude of the responses differed considerably, while the third species (*C. jacea*) hardly responded to exactly the same variation at all. Attempts to predict the population dynamics of poorly studied species from data of well-known cases (see Heppell et al. (2000) for an example with mammals) should therefore be cautiously applied to plants, as our results suggest that generalizations among plant species can be difficult even when they possess comparable life histories.

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Inbreeding depression significantly influences population dynamics: a population-based model for a long-lived perennial herb

F Xavier Picó, Eelke Jongejans, Pedro F Quintana-Ascencio & Hans de Kroon

Summary

Although inbreeding depression usually reduces several fitness traits, the importance of inbreeding depression for the dynamics of plant populations still remains controversial. Assessing the demographic implications of inbreeding depression is essential to develop conservation strategies for plants in fragmented habitats where population sizes tend to be low, the degree of isolation high and inbreeding depression also high. We constructed a simulation model parameterized with demographic and genetic data for the long-lived herb, *Succisa pratensis*, whose populations are highly fragmented in many places across Europe. Demographic data were based on four years of field data from a declining and an increasing population while genetic data were obtained from a greenhouse experiment on inbreeding depression. Inbreeding depression was included into the model by using a relationship between outcrossing probability and the effective population size, and reducing those demographic traits that were significantly affected by inbreeding. Density-dependent recruitment was also incorporated into the model to simulate habitat limitation. Overall, the dynamics of the decreasing population were strongly affected by inbreeding depression, but not by density dependence since the population never reached the carrying capacity. The opposite pattern was exhibited by the increasing population that was less affected by inbreeding depression but limited by density-dependent recruitment.

Keywords: *density dependence, extinction risk, inbreeding depression, matrix population models, perennial plants, population dynamics, stochastic simulations, Succisa pratensis.*

Introduction

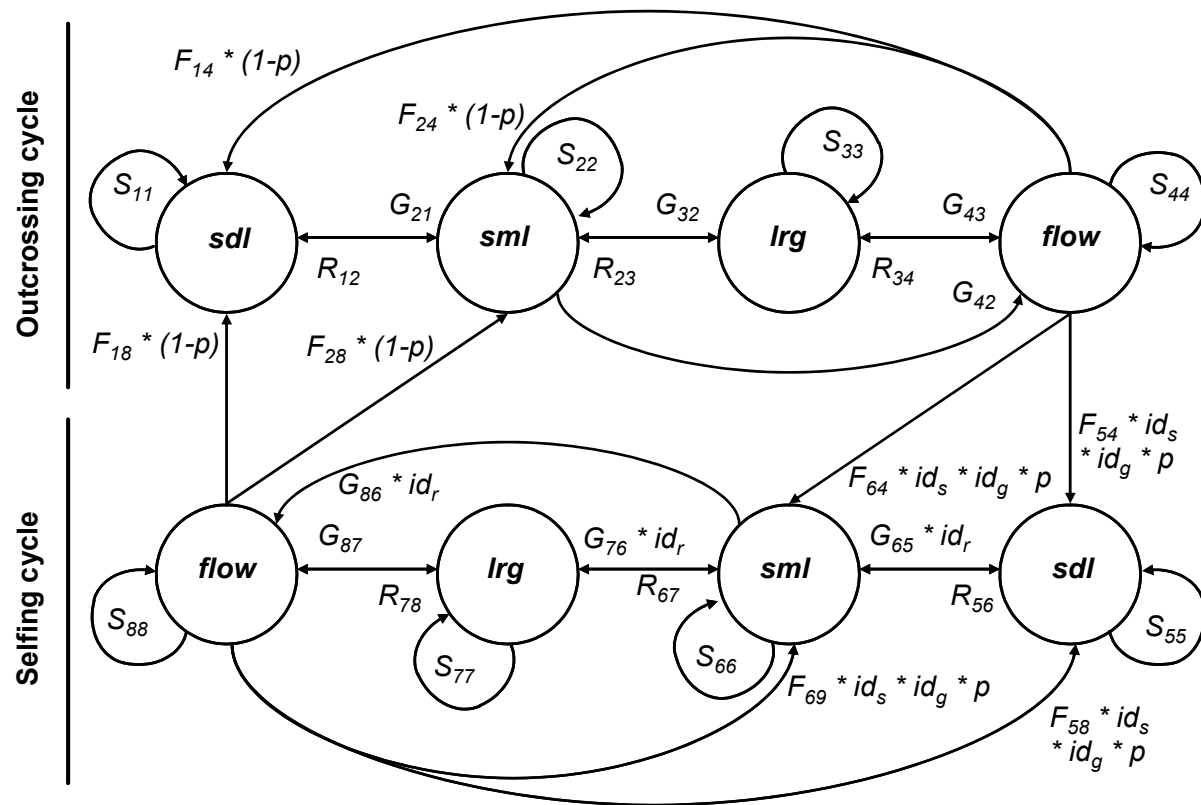
Inbreeding depression, i.e. the reduction in fitness of selfed progeny compared to outcrossed progeny, has traditionally been considered as the main genetic risk

affecting population performance and viability (Soulé 1987). The effects of inbreeding depression on several traits throughout the life cycle of animals and plants have been evidenced by several empirical studies (see Charlesworth & Charlesworth 1999 and references therein). Empirical and modeling work has also shown that the effects of inbreeding depression on important demographic traits may reduce population growth rates and increase extinction probabilities (Newman & Pilson 1997; Saccheri et al. 1998; Bijlsma et al. 2000; Brook et al. 2002; Frankham 2003). However, it has also been argued that the importance of environmental stochasticity and catastrophes could be more important than genetic factors in driving the fate of wild populations because the former occurs far before the latter becomes evident (Lande 1988; Caro & Laurenson 1994).

Such a controversy could be caused by the fact that our empirical knowledge on the demographic implications of inbreeding depression is still very scarce. For example, model systems, such as *Drosophila melanogaster*, has been used to compare the fitness of lines differing in their genetic load (Bijlsma et al. 2000; Reed et al. 2003). Another study created experimental populations of the annual plant *Clarkia pulchella* with high and low levels of inbreeding to investigate the relationship between plant fitness and genetic variability (Newman & Pilson 1997). Other studies have used modeling approaches to simulate population growth of several endangered species assuming that recruitment was always negatively affected by inbreeding depression (Brook et al. 2002; Frankham 2003). Finally, another study has used large-scale long-term surveys of butterfly species to relate extinction risks of natural population with estimates of heterozygosity (Saccheri et al. 1998). Overall, these studies are consistent with the notion that increasing inbreeding depression leads to increasing extinction risks.

Although these studies provided insights into the demographic implications of inbreeding depression, none of them was based on empirical demographic parameters of wild populations combined with effects of inbreeding depression on the life-cycle traits for the species studied. Given that the effects of inbreeding depression on life-cycle traits depend on mating system (Lande & Schemske 1985), and that the contribution of life-cycle traits to population growth rate depends on life history (Silvertown et al. 1993), generalizations on the effects of inbreeding depression on population viability from the scarce current knowledge on this issue are risky. Hence, it becomes essential to develop species-specific conservation plans based on empirical demographic traits including the demographic effects of inbreeding depression on life-cycle traits.

The goal of this study was to assess the demographic implications of inbreeding depression in the perennial herb *Succisa pratensis* (Dipsacaceae). This species faces high degrees of fragmentation in many places across Europe and the species therefore represents a good example of the current status of many common perennial plants across Europe. We constructed a matrix population model based on field data from two populations during five years. We also estimated the effects of inbreeding depression on life-cycle traits throughout the whole life cycle based on a hand-pollination experiment. Because the habitat patches of natural populations of



		stage at time t							
		Outcrossed plants				Selfed plants			
		<i>sdl</i>	<i>sml</i>	<i>lrg</i>	<i>flow</i>	<i>sdl</i>	<i>sml</i>	<i>lrg</i>	<i>flow</i>
stage at time $t+1$	Outcrossed	<i>sdl</i>	S_{11}	R_{12}	-	$F_{14} \cdot (1-p)$	-	-	$F_{18} \cdot (1-p)$
		<i>sml</i>	G_{21}	S_{22}	R_{23}	$F_{24} \cdot (1-p)$	-	-	$F_{28} \cdot (1-p)$
		<i>lrg</i>	-	G_{32}	S_{33}	R_{34}	-	-	-
		<i>flow</i>	-	G_{42}	G_{43}	S_{44}	-	-	-
	Selfed	<i>sdl</i>	-	-	-	$F_{54} \cdot id_s \cdot id_g \cdot p$	S_{55}	R_{56}	$F_{58} \cdot id_s \cdot id_g \cdot p$
		<i>sml</i>	-	-	-	$F_{64} \cdot id_s \cdot id_g \cdot p$	$G_{65} \cdot id_r$	S_{66}	$F_{68} \cdot id_s \cdot id_g \cdot p$
		<i>lrg</i>	-	-	-	-	-	$G_{76} \cdot id_r$	S_{77}
		<i>flow</i>	-	-	-	-	-	$G_{86} \cdot id_r$	G_{87}

Figure 1.

Life cycle graph and population projection matrix including the outcrossing and selfing cycles for *Succisa pratensis*. The four stages are seedlings or very small vegetative rosettes (*sdl*), small vegetative rosettes (*sml*), large vegetative rosettes (*lrg*) and reproductive adults (*flow*). The matrix elements are marked as fecundity from reproductive adults (F_{ij}) or as demographic transitions between stages (R_{ij} , decrease in size; S_{ij} , stasis; G_{ij} , increase in size). Inbreeding depression affects some of the demographic traits (id_s , id_g and id_r for seed set, seed germination, and growth of *sdl* and *sml* when plants enter into the selfing cycle). The probability of producing outcrossed progeny is given by $1-p$, whereas the probability of producing selfed progeny is given by p .

many plant species in Europe are limited in size and limited in the number of available microsites for plants to grow in, a carrying capacity was imposed by a density-dependent recruitment function, which is a conservative way of including habitat limitation. We finally simulated the dynamics of populations differing in the extent of inbreeding depression and density dependence.

Materials and methods

Study species and population sampling

Succisa pratensis is a long-lived, perennial herb widely distributed in heathlands, hay meadows and calcareous fens in Europe. A description of the plant and of its life-history traits can be found elsewhere (Vergeer et al. 2003; Hartemink et al. 2004). Two populations in the Netherlands were selected for this study: Bennekomse Meent (52°00' N, 5°36' E) and Veerslootlanden (52°36' N, 6°08' E), which are part of a study on the dynamics of *S. pratensis* (chapter 5). Both populations are hay meadows and are mown once a year. Populations were monitored from 1999 to 2003 and censused once a year during flowering (July/August and September/October for the two populations respectively). In April 1999, permanent plots (1 × 1 m; five in Bennekomse Meent and three in Veerslootlanden) were laid out and all plants within the plots were tagged and measured. Overall, the number of plants monitored per year per population ranged 157 - 246 plants. For each plant and year, we recorded the status (dead or alive), the number of leaves, the length of the longest leaf, the number of flowering stalks and the number of flowering heads per stalk. Each year, all new seedlings that appeared inside the plots were also counted, measured and tagged.

Experimental crosses

We selected up to 29 adult *S. pratensis* plants (from overall six populations across the Netherlands) that were raised and maintained in the greenhouse of the Botanic Garden of the University of Nijmegen in 1998 for another experiment (Picó et al. *submitted*). In July 2002, for each maternal plant, we created selfed and outcrossed progeny. Two flower heads were self-pollinated with pollen of the same plant and other two were cross-pollinated with pollen of different plants within the same population to obtain selfed and outcrossed progeny, respectively. Prior to hand-pollination, flower heads were emasculated and hand-pollinations were made by gently rubbing one flower head over another one. One head always acted as a pollen donor and the other as a recipient head. All treated flower heads remained bagged during and after hand-pollinations until seed harvesting. In September 2002, we collected all selfed and outcrossed seed produced. We counted and separated filled seeds from the aborted

ones to calculate seed set estimated as the number of filled seeds divided by the total number of seeds produced. Filled seeds were sown in pots (15 × 15 cm) to calculate percent germination per plant and pollination treatment one month after sowing. Up to five seedlings per maternal plant and pollination treatment were randomly selected and individually sown in pots (15 × 15 cm). Three months after transplanting, plant size was recorded as the product of the number of leaves and the length of the longest leaf. The effects of pollination treatment on life-cycle traits of *S. pratensis* were analyzed using one-way ANOVAs, testing the effects of hand-pollination (selfing and outcrossing) on seed set, seed germination and plant growth. We pooled all plants from all populations because population did not have a significant effect on any of the

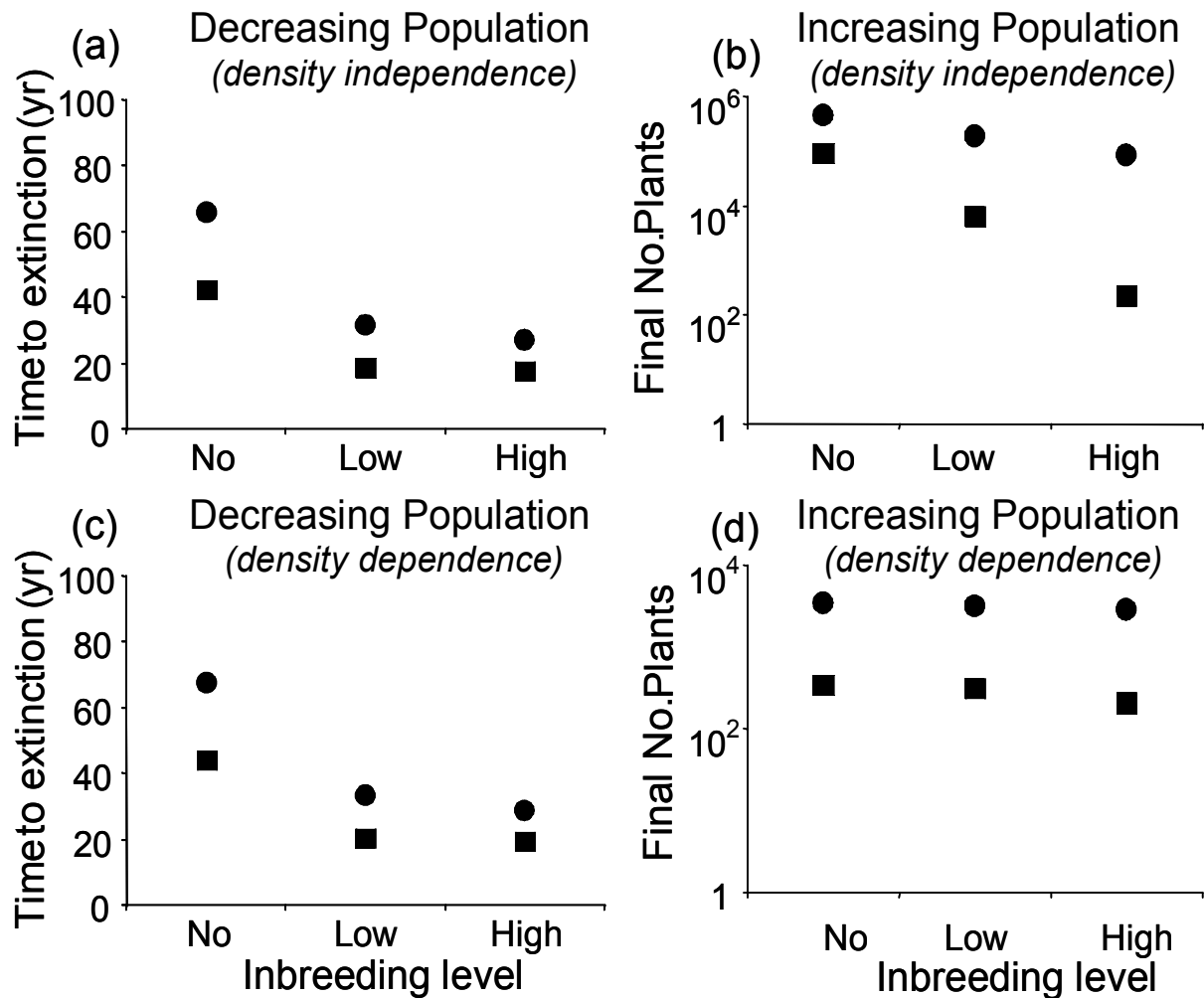


Figure 2.

Stochastic population statistics for the simulated decreasing and increasing *Succisa pratensis* populations in three inbreeding scenarios. Mean \pm SE time to extinction (for the decreasing population) and final number of individuals (for the increasing population) are given. Simulations were performed for small ($N_{initial} = 20$ plants; quadrats) and large ($N_{initial} = 100$ plants; circles) initial population sizes with density independent and density dependent recruitment. SE values are not visible due to their narrow range of variation. Each computation was based on 100 runs.

Table 1.

Summary of demographic vital rates affected by inbreeding depression, the matrix entries modified in the matrices, the mean percent decrease in vital rate obtained from the inbreeding depression experiment, and the percent change in a matrix entry for the low and high inbreeding scenarios used in the simulations. Matrix entries codes as in Fig. 1.

Trait	Matrix entry	Fitness change	Low inbreeding	High inbreeding
Seed set	$F_{54}, F_{64}, F_{58}, F_{68}$	27.3%	$id_s = 20\%$	$id_s = 40\%$
Seed germination	$F_{54}, F_{64}, F_{58}, F_{68}$	13.6%	$id_g = 10\%$	$id_g = 20\%$
Growth of seedlings and small rosettes	G_{65}, G_{76}, G_{86}	46.6%	$id_r = 40\%$	$id_r = 60\%$

traits analyzed ($P > 0.05$ in all analyses; Picó et al. *submitted*). The percentage change in each vital rate due to selfing (an estimate of inbreeding depression, id) was calculated as $id = 1 - (W_s / W_o)$ (Ågren & Schemske 1993). The W_s and W_o are the mean vital rate of selfed and outcrossed progeny, respectively. The mean inbreeding depression for seed set, seed germination and plant growth was calculated as the mean of maternal plant values. Although we did not estimate the effects of inbreeding depression on growth, survival and fecundity of adult stages, other research already indicated that adult stages of *S. pratensis* are not affected by inbreeding depression (Vergeer et al. 2004).

The demographic model

Based on demographic and genetic data, we constructed a demographic model for *S. pratensis* including the effects of inbreeding depression on life-cycle traits. We used size-based Lefkovich population projection matrices (see Caswell 2001 for details). We constructed matrices for four years (from 1999-2000 to 2002-2003) for each population of study.

The matrix model included four size classes: seedlings (*sdI*), small rosettes (*sml*), large rosettes (*lrg*) and reproductive adults (*flow*). The size classification was made largely according to Bühler & Schmid (2001) where the non-flowering classes of *S. pratensis* were differentiated by the maximum leaf length (*sdI*, leaf ≤ 2 cm; *sml*, $2 < \text{leaf} \leq 5$ cm; *lrg*, leaf > 5 cm). Fecundity was defined as the number of seedlings produced per reproductive plant over a year, as *S. pratensis* has no long-term seed bank. Seedling production was estimated as the product of the mean seed production per plant at time t and the seed establishment probability at time $t+1$. Given that some newly recruited seedlings exhibited fast growth rates and reached the *sml* class within one growing season, *sml* production was also estimated as the product of the mean seed production per plant at time t and the *sml* establishment probability at time $t+1$.

Seedling and small rosette establishment probabilities were obtained from a field experiment conducted in 1999 and 2000 in two meadows. In each site and year, 10 plots (5 × 50 cm) with 100 seeds each were set up and the number of seedlings and small rosettes recorded one year after sowing (Chapter 5). The same probabilities were used for all years and temporal variability in *sd* and *sm* production was due to temporal variability in seed production. To keep the model of the life cycle simple we did not account for ramification, which is a rare event anyway in *S. pratensis* (Chapter 5).

Inbreeding depression was included into the demographic model using two criteria. First, the effective population size N_e (i.e. the number of reproductive plants in the population) and the outcrossing probability were proportionally related. The relationship between N_e and inbreeding probability followed a sigmoidal function ($y = [1 + 90 \cdot e^{-0.05 \cdot x}]^{-1}$), where a N_e of 100 corresponded to an outcrossing probability of 0.5. With this function, effective population sizes higher than 250 have no inbreeding depression effects on vital rates. The function was based on a recent study on the relationship between N_e and the genetic variability of *S. pratensis* populations across the Netherlands indicating that the inbreeding coefficient increased with decreasing N_e (Vergeer et al. 2003). Second, those vital rates or components of vital rates that showed significant variability between pollination treatments (Table 1; see the Results section) were modified in the transition matrices. The reduction in demographic transitions due to inbreeding depression was conducted by multiplying the transition by a coefficient *id* that reduced its value. We created two inbreeding scenarios to simulate low and high effects of inbreeding depression by obtaining two sets of matrices with different *id* coefficients to reduce vital rates (Table 1).

The life-cycle graph, and the corresponding population projection matrix, of *S. pratensis* included two cycles (Fig. 1). The matrix of the outcrossing cycle is based on observed population transition matrices. Adult plants can produce selfed seeds which have an establishment rate that is reduced by id_s and id_g as seed set and seed germination are the first components of fecundity affected by inbreeding depression (Table 1). The probability of selfing, p , is a function of N_e at each time step. Seedlings and small rosettes produced from selfing enter into the selfing cycle where their growth is also reduced by id_r (Table 1). Selfed adult plants can also produce two types of progeny, selfed and outcrossed. The selfed progeny will remain within the selfing cycle affected by inbreeding depression whereas the outcrossed progeny will move out to the outcrossing cycle (Fig. 1). It is assumed that seedling and juvenile fates are independent of the inbreeding history of parental plants, and that inbreeding depression does not increase with inbreeding load. We assume that the demographic effects of the proportion of selfed individuals in the population are greater than those caused by the inbreeding history of the individuals.

Matrix analyses and simulations

We carried out three different analyses to assess effects of inbreeding on *S. pratensis*.

Table 2.

Deterministic population growth rates of the decreasing (Veerslootlanden) and increasing (Bennekomse Meent) *Succisa pratensis* populations for each year of study and of resulting matrices after modifying their elements (see Table 1) due to low and high inbreeding depression effects. The change (%) in population growth rate is indicated in parentheses. The mean (SE) population growth rates over years are also given.

Decreasing population			
Year	Observed matrices	Low inbreeding	High inbreeding
1999 - 2000	0.901	0.808 (10.32)	0.760 (15.65)
2000 - 2001	1.060	0.937 (11.60)	0.885 (16.51)
2001 - 2002	0.909	0.859 (5.50)	0.839 (7.70)
2002 - 2003	0.854	0.833 (2.46)	0.833 (2.46)
Mean \pm SE	0.931 \pm 0.052	0.859 \pm 0.032	0.829 \pm 0.030
Increasing population			
Year	Observed matrices	Low inbreeding	High inbreeding
1999 - 2000	1.290	1.124 (12.87)	1.032 (20.00)
2000 - 2001	1.205	1.075 (10.79)	1.005 (16.60)
2001 - 2002	1.312	1.162 (11.43)	1.077 (17.91)
2002 - 2003	1.295	1.134 (12.43)	1.048 (19.07)
Mean \pm SE	1.275 \pm 0.028	1.124 \pm 0.021	1.043 \pm 0.017

First, for each population and year, we computed deterministic population growth rates, λ , for the observed matrices, and for the sets of matrices with low and high inbreeding depression. We performed elasticity analysis on the observed population matrices to determine the relative contribution of life-cycle traits to the population growth rate (de Kroon et al. 1986; Caswell 2001). Second, for each population, we conducted stochastic simulations on *S. pratensis* populations including environmental stochasticity into the model by randomly selecting a matrix from the pool of the four observed matrices at each time step (Caswell 2001). We ran the model three times: one using the observed set of matrices (no inbreeding; $p = 0$), another using the set of matrices modified with low inbreeding depression, and another using the set of matrices modified with high inbreeding depression (see Table 1 for id values). In the latter two simulations, p varied as a function of N_e at each time step of the simulation. For each simulated population, we computed the mean final number of individuals or the mean time to extinction computed over 100 runs. A population went extinct when the total number of individuals in the population was < 1 . Each simulation run lasted for 30 years. The effects of initial population size were incorporated by simulating initial population sizes of 20 (with selfing probability $p = 0.97$) and 100 ($p = 0.5$) reproductive individuals.

Finally we examined the joint effects of inbreeding depression and density dependence on vital rates. Assuming that density dependence mostly affects recruitment and growth of pre-adult classes (de Kroon et al. 1987; Gillman et al. 1993;

Crawley 1997: 491), all demographic transitions involving *sdl* (i.e. S_{11} and G_{12}) and *sml* (i.e. G_{21} , S_{22} , G_{23} and G_{24}) were multiplied by d that decreases the matrix entry as total population size N increases. Given that we did not have empirical data to estimate the density dependence function for *S. pratensis*, we used another sigmoidal function that related N and d . The sigmoidal function had the form $y = (1 + 4000 \cdot e^{-0.04 \cdot x})^{-1}$. With this function, populations with more than 300 individuals were strongly affected by density dependent effects.

Results

Inbreeding depression significantly affected seed set ($F_{1,65} = 5.89$, $P = 0.018$), percentage germination ($F_{1,33} = 5.47$, $P = 0.026$) and growth of pre-adult plants ($F_{1,19} = 11.75$, $P = 0.003$). The decrease in seed set, percent germination and growth of seedlings and small rosettes due to inbreeding depression ranged 14 - 47% (Table 1).

Like other long-lived perennials, *S. pratensis* exhibited high survival rates (> 67.5% for all size classes) and low recruitment rates (range 0.26 - 2.3 and 0.19 - 1.4 seedlings and small rosettes per reproductive plant, respectively). On average stasis and retrogression accumulated up to 42 - 84%, growth 14 - 43% and fecundity 2.2 - 15% of the total elasticity. Each demographic transitions affected by inbreeding depression (fecundity and growth of pre-adult individuals) accounted for less than 7% that altogether summed up to 28.9% of the total elasticity. Population growth rates of the decreasing and increasing population of study were 0.93 and 1.28, respectively (Table 2). When inbreeding depression was included into the matrices, deterministic population growth rates of the decreasing population were reduced 2.5 - 11.6% and 2.5 - 16.5% for the low and high inbreeding scenarios, respectively (Table 2). The reduction due to inbreeding depression was more pronounced for the increasing population with reductions in the deterministic population growth rates of 10.8 - 12.9% and 16.6 - 20.0% for the low and high inbreeding scenarios, respectively (Table 2).

Density-independent stochastic simulations indicated that for the decreasing population the mean time to extinction was shortened about 50% under the low and high inbreeding scenarios as compared to the no inbreeding scenario (Fig. 2a). For the increasing population, inbreeding also had a dramatic effect by reducing population growth with more than 60%, though simulated populations still exhibited an increasing dynamics (Fig. 2b). The effects of small initial population size were more dramatic for the increasing than for the decreasing population (Fig. 2a and 2b).

For the decreasing population density-dependent stochastic simulations yielded the same results as those obtained from the density-independent stochastic simulations (Fig. 2c), due to the fact that recruitment was hardly affected by density dependence at low population sizes. For the increasing population however, density dependence strongly reduced population growth. Inbreeding depression decreased population growth slightly in the increasing population with density dependency (Fig.

2d). This was true for the small initial population but not for the large population where inbreeding had hardly any effect at all in population growth (Fig. 2d). In fact, the increasing population grew up to the carrying capacity determined by the density dependent function where inbreeding rates were very low.

Discussion

The results showed that inbreeding depression has important demographic consequences for the *Succisa pratensis* populations studied. The increasing and decreasing populations generally showed important reductions in growth rates and mean time to extinction, respectively. Overall, our results agree with other studies that also indicated that inbreeding depression can strongly affect population dynamics increasing the risk of extinction (Halley & Manasse 1993; Newman & Pilson 1997; Saccheri et al. 1998; Bijlsma et al. 2000; Groom & Preuninger 2000; Tanaka 2000; Brook et al. 2002; Frankham 2003). However, our study does avoid a common pitfall of other demographic genetic models (Alvarez-Buylla et al. 1996; Oostermeijer et al. 2002) by fully parameterizing the model with field data for demographic attributes and with hand-pollination data for inbreeding depression effects on life-cycle traits. It can be misleading to model the population dynamics of a particular species based on detailed data for only one of the two components required (either demographic data or inbreeding data) while using rough estimations for the other.

In the case of assessing a species conservational status, using detailed demographic and inbreeding data is essential to obtain accurate results. Our simulations for *S. pratensis* clearly indicated that increasing populations can cope with inbreeding whereas decreasing populations become more prone to extinction due to inbreeding depression. *Succisa pratensis* has a long lifespan that permits extant populations to persist for long time in a given area (i.e. remnant dynamics; Eriksson 1996). Nonetheless, our results suggest that inbreeding depression can strongly reduce the species population fitness especially for decreasing and small populations. It must also be emphasized that such a reduction in population performance was accounted for by components of life-cycle traits affected by inbreeding depression that altogether accumulated less than 30% of the total elasticity. This means that if other life-cycle traits more important for the persistence of *S. pratensis* populations (e.g. adult survival) were affected by inbreeding depression, the demographic implications of inbreeding depression would have even been more dramatic. For this reason, identifying the demographic traits that are truly affected by inbreeding depression and quantifying their proportional change become essential to accurately estimate the demographic implications of inbreeding depression.

Changes in population size represent the mechanism that tunes the effects of inbreeding depression on population behavior, as the inbreeding coefficient significantly increases with decreasing population size in *S. pratensis* (Vergeer et al.

2003). We have included two sources of variation that affected population size and subsequently the extent of inbreeding depression: first, environmental stochasticity due to year-to-year variation in environmental conditions and second, density-dependent recruitment that limits population growth mimicking habitat size limitation. Other simulation studies that included environmental stochasticity, carrying capacity and inbreeding depression (Halley & Manasse 1993) also stressed the importance of the interaction between these factors. In general, the effects of inbreeding depression, which depend on the relationship between population size and outcrossing probability, depend in turn on the carrying capacity of the system. Our results showed that the decreasing population was more affected by inbreeding depression than by density dependence since the population never reached the carrying capacity. The opposite pattern was exhibited by the increasing population that was less affected by inbreeding depression but limited by density-dependent recruitment.

Density dependence and inbreeding depression do not interact as long as the habitat size is large enough to harbor sufficient numbers of individuals to eliminate the risk of inbreeding depression. In contrast to habitat size, habitat quality can interact with inbreeding depression. It has been shown that the performance of *S. pratensis* populations can be as much affected by habitat quality as by inbreeding depression (Vergeer et al. 2003). Deteriorating habitat quality leads to decreasing population size as well as increasing inbreeding and inbreeding depression, factors that act in concert increasing extinction risks (Vergeer et al. 2003). Our simulations empirically support this view showing how decreasing populations, presumably due to poor habitat conditions, can enter faster into the so-called 'extinction vortex' (Gilpin & Soulé 1986) due to the effects of inbreeding depression on demographic traits.

Theoretically, the extinction of small populations could be prevented by the genetic rescue effect, that is, the arrival of immigrants from surrounding populations that could replenish genetic variation and reduce inbreeding depression (Ingvarsson 2001). In the case of *S. pratensis* as well as many other plant species in Europe, the extent of fragmentation is high (Bakker & Berendse 1999) resulting in reduced colonization capacity of extant populations (Soons & Heil 2002). This scenario limits the effectiveness of the genetic rescue effect and poses a serious threat to the survival of many plant species in fragmented landscapes. It has been suggested that purging of deleterious mutations might reduce the effects of inbreeding depression (Charlesworth & Charlesworth 1999), so that small populations could increase their performance under high inbreeding levels. However, reviews to assess the extent of purging have revealed that the importance of purging is controversial (Byers & Waller 1999; Miller & Hedrick 2001) and that purging is probably not strong enough to be of practical use in eliminating inbreeding depression (Dudash et al. 1997; Koelewijn 1998; Lacy & Ballou 1998; Frankham et al. 2001). Although the link between increasing genetic variation, mean fitness and population persistence is still largely unknown (Ingvarsson 2001), studies focusing on conservation measures to prevent *S. pratensis* population decline should include habitat restoration (Vergeer et al. 2003) as well as reintroduction of individuals from other populations to the loss of genetic variation and inbreeding depression (Vergeer et al. 2004).

Acknowledgments

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Effects of increased productivity on the population dynamics of the endangered, clonal herb *Cirsium dissectum*

Eelke Jongejans, Natasha de Vere & Hans de Kroon

Summary

Plant species with rhizomatous runners have the potential to effectively exploit and occupy their habitat. But even in this group rare plants are not uncommon, as is exemplified by *Cirsium dissectum*, a characteristic clonal herb of nutrient-poor, wet grasslands, which is endangered by habitat destruction and nutrient enrichment. In this paper we aim to investigate how rosettes of *C. dissectum* respond nutrient enrichment and what the consequences are for the population dynamics. A previous experiment revealed that this species increases its allocation to seed production and not to vegetative biomass when plots with this species and a common grass competitor were fertilized. Here we further analyze these experimental allocation patterns and data on excavated plants in seven sites of increasing productivity, for underlying mechanisms. Both sexual and clonal reproduction increased with rosette size. An additional greenhouse experiment on rhizome formation confirmed the size-dependency of clonal propagation and showed no inherent differences between plants from different sites. Demographic data were recorded in permanent plots in three sites from 1999 till 2003. Elevated clonal propagation and rosette mortality were found in both the fertilized garden plots and the most productive site. The combination of faster rosette growth and higher biomass turn-over therefore explained the observed trend of higher percentages of flowering rosettes at high nutrient availability. Furthermore, increased rosette mortality rates may cause decreasing genetic diversity in the already small, remnant populations of *C. dissectum*, as seedling establishment seems to be limited to bare soil.

Keywords: *Cirsium dissectum*; clonal propagation; demography; life table response experiments; nutrient availability; projection matrix model; rosette turn-over; sexual reproductive allocation; size dependent reproduction

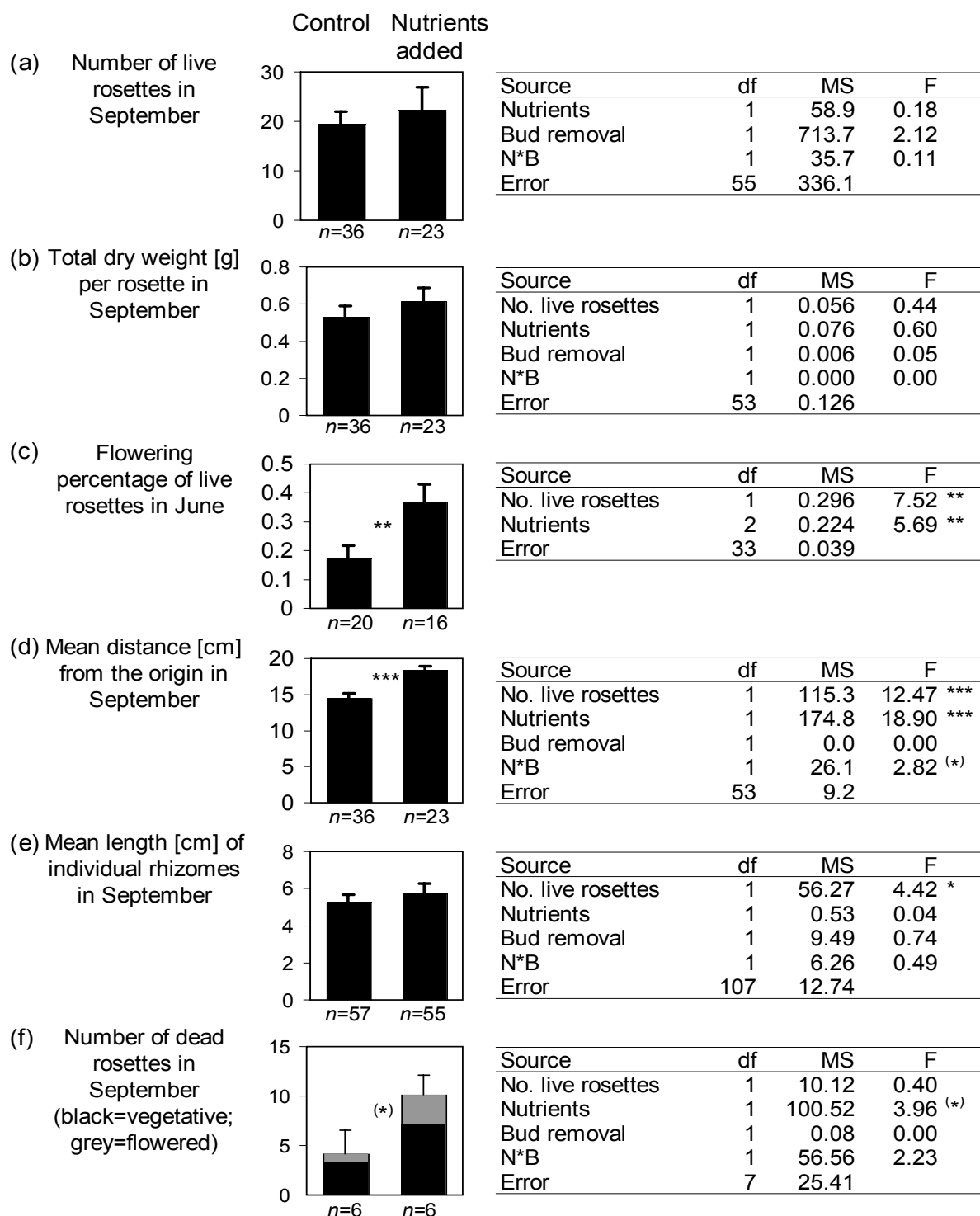
Introduction

Clonal growth by long stolons or rhizomes can be a successful life history strategy as runners may be an effective way to exploit heterogeneous resources or to rapidly occupy space (Eriksson 1986; de Kroon & Knops 1990). This group of plant species contains notorious examples such as *Phragmites australis*, *Pteridium aquilinum* and *Brachypodium pinnatum* which can quickly attain dominance through clonal growth under suitable conditions and may form monocultures that hold up succession (de Kroon & Bobbink 1997; Marrs et al. 2000). But runners may not be a guarantee for success, since the percentage of rare species among clonal plants with rhizomes longer than 10 cm is equal to the percentage for other types of clonal growth (Klimeš & Klimesová 2000). This paradox of plant rarity and long runners needs more clarification.

An example of a rhizomatous but rare (Red List) species is the Meadow thistle, *Cirsium dissectum*. This inhabitant of rather nutrient-poor, species-rich, wet grasslands has an Atlantic distribution in Northwest Europe. Habitat destruction and habitat fragmentation, desiccation, acidification and nutrient enrichment have led to reductions in the number and size of *C. dissectum* populations (Saunders et al. 1991; Tilman et al. 1994; Roem & Berendse 2000; Soons et al. 2003). This early-successional grassland species is replaced by more competitive plants and nutrient enrichment speeds up this process of succession in grasslands.

In general clonal plants have been hypothesized to respond to increased vegetation biomass by increasing either sexual reproduction or clonal propagation depending on their life history strategies, on the expected relative gain of increasing either way of reproduction and on the detectability of environmental cues (Abrahamson 1980; Loehle 1987). To test this hypothesis for *C. dissectum* an allocation experiment was performed in which this species was grown together with the tall grass *Molinia caerulea* in nutrient enriched plots and unfertilized control plots (Chapter 3). After three years the grass had benefitted from the extra nutrients, while the genet mortality rate of *C. dissectum* was higher in fertilized plots. A higher percentage of the clonal offspring of the surviving *C. dissectum* plants flowered in the fertilized plots compared to the plants in the control plots, while the number of living rosettes and total biomass was not different. This increased sexual reproductive allocation can be seen as a strategy of escape in space when seeds may disperse to more favorable sites. It is unclear however what mechanism caused this shift in allocation pattern or whether similar patterns can be found in natural populations over a gradient of productivity. Genetic differences between populations however may potentially also be a source of variation in sexual reproductive allocation. Besides, the importance of shifts in allocation depends on their impact on the dynamics of natural populations.

In this paper we study the role of clonal growth and seed production in the population dynamics of *C. dissectum* in response to nutrient enrichment. To meet the above-mentioned objectives we use a set of experiments and field observations: 1) To

**Figure 1.**

Overview of the effect of nutrient addition on *Cirsium dissectum* in the allocation experiment: (a) number of rosettes alive (i.e. with green leaves) at harvest, September 2002, (b) the total dry weight [g] of *C. dissectum* per plot at harvest after dividing by the number of rosettes, (c) the percentage of rosettes in that flowered June 2002 (of the plots without bud removal only,) (d) the mean distance [cm] of the rosettes at harvest measured from the position of the first, planted rosette at the beginning of the experiment, (e) the mean length [cm] of all individual rhizomes that connected two rosettes in 12 randomly selected plots in which the rhizomes were revealed, and (f) the number of rosettes in those 12 plots that were dead. ANOVA results on these six parameters are given on the right. The number of live rosettes at the time of measuring is used as covariate, nutrient addition and bud removal as fixed factors. N*B is their interaction. (*) $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

better understand the mechanism of the observed increased seed production, the allocation experiment is further analyzed to investigate the relation with of clonal growth and rosette mortality. 2) To check if these allocation shifts also occur in natural populations, we compare plant sizes, sexual reproduction and clonal propagation between seven field populations over a gradient of productivity. 3) To test whether differences in growth patterns are genetic or plastic we perform a small greenhouse experiment with plants from five different populations. 4) And finally we study the population dynamics of *C. dissectum* for four years in three sites with contrasting productivity to quantify the contributions of rosette survival, sexual reproduction and clonal propagation to population growth.

Materials and methods

Study system

Meadow thistle, *Cirsium dissectum* (L.) Hill Asteraceae, is a rhizomatous herb that forms rosettes with up to five softly prickled leaves, but mostly only two or three. In winter the above-ground rosette is reduced to one or two small, fleshy leaves. Flowering stems are formed apically. During stem elongation rosette leaves are raised up to half way the 60 cm tall flowering stem. After seed set the rosette dies off. Locally it can form dense stands by clonal propagation. *Cirsium dissectum* is mostly restricted to oligotrophic wet grasslands and heathlands over its entire distribution in Atlantic North-west Europe. These habitats have declined substantially in number and extent (Kay & John 1994; Buck-Sorlin & Weeda 2000). Fifty-seven percent of the *C. dissectum* populations in Germany are estimated to have become extinct since 1930, and many surviving populations were decimated in size, due to drainage and fertilization of the sites they inhabit (Buck-Sorlin 1993). *Cirsium dissectum* plants are rather sensitive to the effects of acidification (de Graaf et al. 1997; de Graaf et al. 1998; Lucassen et al. 2003). The status of the Dutch *C. dissectum* populations is currently being investigated by the floristic foundation FLORON (Rossenaar & Groen 2003).

Allocation experiment

To investigate the effects of nutrient enrichment on allocation to sexual reproduction we performed a three-year experiment in a nutrient-poor, sandy garden of Wageningen University (Chapter 3). One *C. dissectum* rosette was planted between six clumps of *Molinia caerulea* to mimic a grassland containing *C. dissectum* and plants that rapidly increase biomass when more nutrients are available. Half of the plots were enriched with the equivalent of 120 kg N ha⁻¹ yr⁻¹ during the second and

third year of the experiment, while the other half received no fertilization. To quantify the costs of sexual reproduction on vegetative growth the flower buds were continuously removed from half of the plants in both the nutrient addition and the control group. More details on the methods and the main results on the number of rosettes and the biomass of different plant parts are presented in chapter 3.

At the end of the third growing season, just before the harvesting of the experiment we mapped the position of all *C. dissectum* rosettes in the circular plots. In order to study the degree of spatial spread from the initial rosette position, the distance of each rosette to the geometrical centre of the six *M. caerulea* clumps was determined on the maps. Detailed observations were made on the below-ground rhizome network in three randomly selected plots in each of the four groups (nutrient addition and bud removal treatment combinations). High-pressure air was used to remove the soil around the rosettes to reveal rhizomes and decaying rosettes without leaves. These leafless rosettes and rhizomes were also mapped.

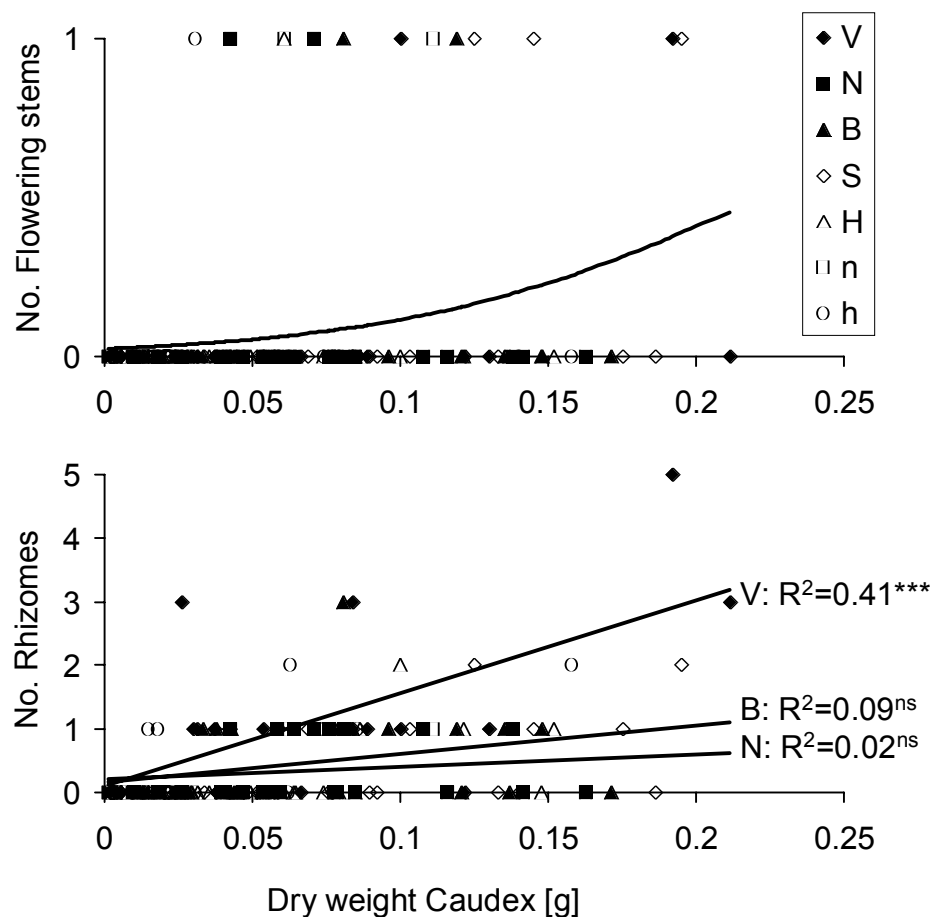


Figure 2.

Relationships between flowering and the number of produced rhizomes with the dry weight [g] of the caudex of excavated rosettes in the field survey. The 159 rosettes were randomly selected (see methods) in seven sites: Nijkampse heide (h and H), Stelkampsveld (S), Bennekomse meent (B), Konijnendijk (n and N) and Veerslootlanden (V), in which non-capital letters denote sod-cut areas. The logistic regression on flowering was performed on all data together: $y = (\exp(16.6 \cdot x - 3.70)) / (1 + \exp(16.6 \cdot x - 3.70))$. The linear regression on rhizome number was performed for each site separately. Four regression lines are not shown: they are intermediate to V and B, and are all significant. ** $p < 0.01$; *** $p < 0.001$.

Site survey

The relationship between rosette size, flowering probability, rhizome formation, and site characteristics was studied in five grasslands. The sites are: Nijkampse heide (H) (52°02' N, 6°33' E), Stelkampsveld (S) (52°07' N, 6°29' E), Bennekomse Meent (B) (52°00' N, 5°36' E), Konijnendijk (N) (52°02' N, 6°26' E), and Veerslootlanden (V) (52°36' N, 6°08' E). All sites are mown once per year after which hay is removed. The population in site V was mown twice in some years in order to halt encroachment of Common reed, *Phragmites australis*. The topsoil of parts of the grasslands H and N had been removed to reduce soil fertility (sod-cutting) in 1997. These sod-cut areas are referred to as h and n.

Between July 26th and August 3rd, 2001, five 50 by 50 cm square plots were randomly laid out in each of the seven areas. For the non-sod cut areas plots had to contain a minimum of five rosettes, this was reduced to a minimum of two rosettes for the sod-cut areas since there *C. dissectum* density was lower. Plots containing less than the minimum number of rosettes were omitted and new plots chosen randomly.

Within each plot we estimated the height of the vegetation (without flowering stalks) and the percentage vegetation cover. The positions of all *C. dissectum* rosettes were mapped with an accuracy of 5 mm. The size of each rosette was determined as the number of leaves and their maximum length. For flowering individuals the height of the stem was measured. Five rosettes (and at least two in the sod cut areas) per plot were randomly selected and carefully excavated. Rhizomes connected to these rosettes were followed to their destination, which was outside the plots in some cases. The maximum depth and the distances between connected rosettes were measured for each excavated rhizome. The vegetation in the plot was clipped at a height of four centimeters above the soil surface, and weighed after being dried for 48 hours at 70°C. The average thickness of the humus layer was determined from three soil cores next to each plot.

Rhizome formation experiment

In order to investigate possible genetic differences in growth rate and rhizome formation, plants from the five sites were grown under equal conditions. During the first half of May 2001 small rosettes with two leaves were randomly selected and dug out from each site. These rosettes were at least one winter old as clonal propagation does not start before May. The roots were rinsed with water, and one rosette placed in the middle of a 34 by 34 cm square and 15 cm deep container filled with nutrient-poor sandy soil. Half of the containers were seeded with the grass *Agrostis capillaris* to mimic the difference between sod-cut areas and grasslands. The *A. capillaris* seeds established readily, and was cut regularly at 4 cm height to avoid shading the *C. dissectum* rosettes and to focus on below-ground competition only. The experiment was performed in an open, unheated greenhouse roofed with transparent foil. The containers were watered daily. Every fortnight the containers were weighed and their

weight was brought back to the original 14,000 g by watering. The experiment had a full block design with 60 containers: five site origins, two treatments and six replicates.

The experiment lasted five months with the final, block-wise harvest between October 23rd and November 8th. The number of clonal offspring and the distance between new rosettes and the original one were determined. For each container the soil was rinsed and the *C. dissectum* plants separated from the *A. capillaris*. The biomass was determined for each species after drying for 48 hours at 70°C.

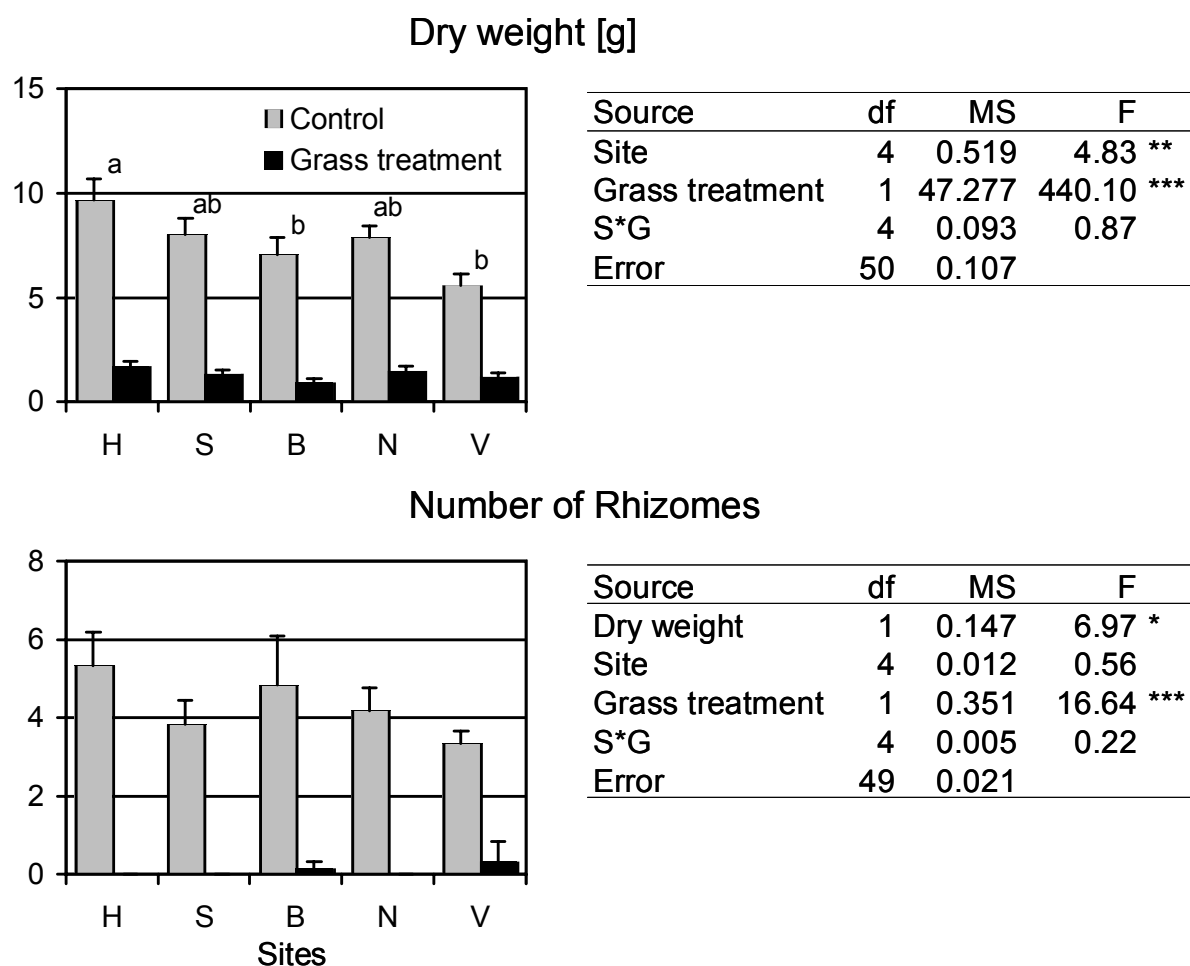


Figure 3.

The dry weight [g] and number of rhizomes per *Cirsium dissectum* rosette in an open greenhouse experiment in which rosettes from different sites were grown in pots with (grass treatment) or without (control) a competitor, *Agrostis capillaris*. The error bars are standard errors. Different letters denote significant difference in dry weight between sites (post-hoc Tukey test). The variation in rhizome number is successfully explained by both the grass treatment as factor and plant dry weight as covariate, but site of origin was not a significant factor. Sites were in order of increasing productivity: Nijkampse heide (H), Stelkampsveld (S), Bennekomse meent (B), Konijnendijk (N) and Veerslootlanden (V).

Demography

To study the natural population dynamics of *C. dissectum* we established permanent plots in which all rosettes were followed from 1999 to 2003. The 1 by 1 m² permanent plots were established in April 1999 at three sites: five in B, six in N and two in V. Each year in June, when *C. dissectum* flowers, all rosettes in the permanent plots were mapped and measured: we counted the number of rosette leaves and determined maximum leaf length. If a rosette had produced a flowering stem, the number of flower heads was counted.

A combination of plant size, flowering status, and the formation of side-rosettes was used to classify rosettes. We distinguished six classes (Fig. 1 of Chapter 1):

- 1 Seedlings (*sdl*) are tiny rosettes with no leaves longer than 2 cm.
- 2 Small vegetative rosettes (*sml*): larger than seedlings but with no leaves longer than 12.5 cm.
- 3 Large vegetative rosettes (*lrg*) have longer leaves than small rosettes.
- 4 Flowering rosettes (*flow*) are grouped irrespective of rosette size.
- 5 Vegetative clonal offspring (*cl.v*) are new rosettes formed by rhizomes since the census of the preceding year and do not flower.
- 6 Flowering clonal offspring (*cl.f*) are new rosettes formed by rhizomes since the census of the preceding year and flower in the year in which they are formed.

Seedling establishment was studied in a seed addition experiment since the number of seedlings in the permanent plots was very low. Fifty seeds were added to ten 5x50cm plots in B, N, and O in November 1999 and 2000 (Chapter 4). The number of seedlings and small rosettes just before and 12 months after seed addition was compared to control plots. Seedling and small rosette establishment per seed were calculated as follows:

$$p_{sdl} = \frac{(N_{sdl,t=1}^{SA} - N_{sdl,t=0}^{SA}) - (N_{sdl,t=1}^{NS} - N_{sdl,t=0}^{NS})}{N_{seeds}^{SA}} \quad (1)$$

$$p_{sml} = \frac{(N_{sml,t=1}^{SA} - N_{sml,t=0}^{SA}) - (N_{sml,t=1}^{NS} - N_{sml,t=0}^{NS})}{N_{seeds}^{SA}} \quad (2)$$

where N is the number of seeds, seedlings (*sdl*) or small rosettes (*sml*) in the seed additions plots (SA) and control plots (NS) at the beginning ($t=0$) of the experiment or one year later ($t=1$).

Matrix parameterization

The demographic data from the permanent plots and the seed addition experiment were used to parameterize 6 by 6 projection matrices of the form A_{ij} in which a_{ij} represents the transition element from the j^{th} category in year t to the i^{th} category in

year $t+1$. The 36 matrix elements were classified as different life history components: fecundity (F), growth (G), clonal propagation (C), survival of clonal offspring (T), stasis (S) and retrogression (R) (Table 3). Growth is defined as the transition of a smaller or vegetative stage class to a larger or flowering stage class by a surviving rosette. Stasis means that a rosette remains in the same class and rosettes that retrogress survive but decrease in size. Fecundity of the flowering plants was calculated as the product of the establishment probabilities per seed, the average seed production of the average flowering rosette in each population, and the mean number of observed flower heads per flowering rosette in a population in year t .

Three important assumptions were made during the construction of the population matrices. Firstly, since only a small number of seedlings were present in both the permanent plots and the seed addition experiment, seedling fates, S_{11} and G_{21} , were estimated conservatively high at 55% however (comparable to that of *Hypochaeris radicata*, another Asteraceae species; Chapter 5). Secondly, because rhizomes are invisible without excavations we could not determine which rosette in year t clonally produced which new rosette in year $t+1$. Therefore we assigned new clonal offspring to old rosettes of either of the stage classes *sml*, *lrg*, *flow*, *cl.v* or *cl.f*, according to a probability function that was based on two factors: the proportion of all rosettes in year t belonging to a certain class, and the average number of rhizomes produced by either a vegetative (*sml*, *lrg* and *cl.v*) or a flowering (*flow* and *cl.f*) rosette. The latter was calculated for each population separately, based on the excavated plants in the site survey. No distinction was made in the rhizome production of different

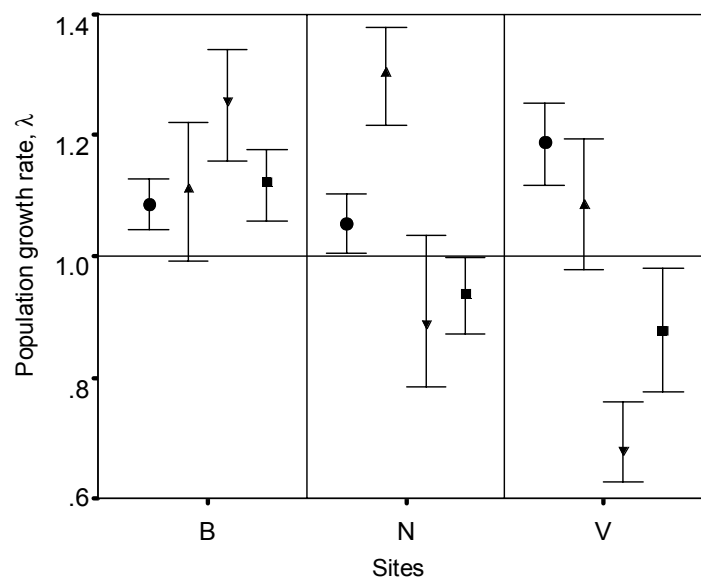


Figure 4.

Projected population growth rate, λ , and bootstrapped 95% confidence intervals for all *Cirsium dissectum* matrices. Site and year codes: Bennekomse Meent (B), Konijnendijk (N), Veerslootlanden (V), ● = 1999-2000, ▲ = 2000-2001, ▼ = 2001-2002 and ■ = 2002-2003.

vegetative size classes as there was no significant relationship between the number of produced rhizomes and plant size classified as either *sml* or *lrg*. Berg (2002) made similar assumptions in his *Oxalis acetosella* model. Thirdly, some transition probabilities had to be estimated as some data were absent or not sufficient: in the first year (1999 to 2000) it was not possible to observe fates of clonal offspring formed in the previous year, because no observations were made in 1998. This leads to empty matrix elements of new clonally-produced rosettes for that year. Similar problems occurred when a stage class contained less than six rosettes at time t . In these cases the transition probabilities from a class in a certain year were estimated from all observations over the years for that class in the same population. Apart from the *cl.v* and *cl.f* classes in the first year, observation numbers were too small in only 13% of the cases.

Matrix analysis

The 12 (three sites for four years) constructed projection matrices were analyzed using the Matlab student edition (1996). For each matrix the dominant eigenvalue and the accompanying right eigenvector were used as the projected population growth rate, λ , and the stable-state distribution respectively (Caswell 2001). Furthermore, we determined the elasticity value e_{ij} of each matrix element a_{ij} of each population transition matrix (de Kroon et al. 1986; de Kroon et al. 2000):

$$e_{ij} = \frac{a_{ij}}{\lambda} \frac{\partial \lambda}{\partial a_{ij}} \quad (3)$$

Elasticity values quantify the relative contribution of each element to λ and sum to one. To construct 95% confidence intervals for the lambdas, we applied the bootstrapping method by resampling the data set of observed rosette fates of each matrix 3,000 times (Efron 1982; Kalisz & McPeck 1992; Caswell 2001) in which the survival (T , G , S , and R) and ramification (C) observations were resampled for all classes separately. New sexual reproduction (F) elements were calculated after resampling the data set of number of flower heads of the observed flowering rosettes. The upper and lower limits of the 95% confidence intervals were adjusted for small deviations between the mean λ of the 3000 newly constructed matrices and the λ of the original matrix (Caswell 2001 p. 306).

To decompose variation in λ we applied factorial life table response experiments (LTRE). The model for such a decomposition analysis with two factors within a species is (Horvitz et al. 1997; Caswell 2001):

$$\lambda^{(mn)} \cong \lambda^{(\cdot\cdot)} + \alpha^m + \beta^n + (\alpha\beta)^{(mn)} \quad (4)$$

in which a given λ of the m^{th} site and n^{th} year is written as the sum of the dominant eigenvalue of the mean of all matrices of a species, $\lambda^{(\cdot\cdot)}$, the main effect of the m^{th} site,

α^m , the main effect of the n^{th} year, β^n , and the residual ‘interaction’ effect, $\alpha\beta^{(mn)}$. The main effects can be estimated filling in this equation first for each level of the main effects separately, while ignoring the interaction term. The main and interaction effects can now be decomposed into contributions from each matrix element (Caswell 2001):

$$\tilde{\alpha}^m = \sum_{i,j} (a_{ij}^{(m\cdot)} - a_{ij}^{(\cdot\cdot)}) \frac{\partial \lambda}{\partial a_{ij}} \bigg|_{\frac{1}{2}(A^{(m\cdot)} + A^{(\cdot\cdot)})} \quad (5)$$

$$\tilde{\beta}^n = \sum_{i,j} (a_{ij}^{(\cdot n)} - a_{ij}^{(\cdot\cdot)}) \frac{\partial \lambda}{\partial a_{ij}} \bigg|_{\frac{1}{2}(A^{(\cdot n)} + A^{(\cdot\cdot)})} \quad (6)$$

$$\left(\tilde{\alpha\beta} \right)^{(mn)} = \sum_{i,j} (a_{ij}^{(mn)} - a_{ij}^{(\cdot\cdot)}) \frac{\partial \lambda}{\partial a_{ij}} \bigg|_{\frac{1}{2}(A^{(mn)} + A^{(\cdot\cdot)})} - \tilde{\alpha}^m - \tilde{\beta}^n \quad (7)$$

in which differences of each matrix element with the corresponding matrix element of the overall mean matrix, $A^{(\cdot\cdot)}$, are multiplied by the sensitivity values of the matrix

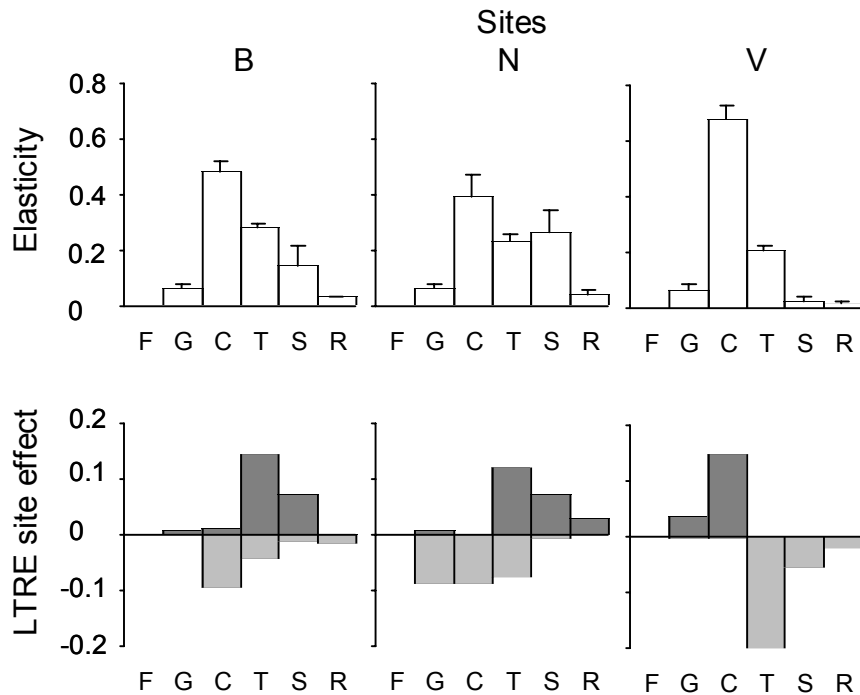


Figure 5.

Elasticity values (mean and standard error) and site effects in the decomposition (LTRE) of variation in population growth rate, λ , of three *Cirsium dissectum* populations: Bennekomse meent (B), Konijnendijk (N) and Veerslootlanden (V). Elasticity values and positive and negative contributions of matrix elements are separately grouped by life history component: fecundity (F), growth (G), clonal reproduction (C), survival of clonal offspring (T), stasis (S) and retrogression (R). Mean λ is 1.141, 1.043 and 0.968, and netto LTRE site effects, α^m , is +0.072, -0.025 and -0.097 for sites B, N and V respectively.

halfway between the matrix of interest and the overall mean matrix. The resulting contribution matrices of the main and interaction effects contain both positive and negative values, which we summed separately for each of the six life history components (*F*, *G*, *C*, *T*, *S* and *R*).

Results

Allocation experiment

The nutrient addition treatment increased the biomass of the tall grass *Molinia caerulea*, but not the biomass of *Cirsium dissectum* (Chapter 3). Nutrient enrichment also had no effect on the dry weight of individual rosettes (Fig. 1b). The percentage of those rosettes that flowered however was significantly higher in the plots that received nutrients (Fig. 1c). The rosettes in the nutrient-rich plots were also significantly further away from the position of the first, planted rosette (Fig. 1d). This could not be explained by differential rhizome length, as this parameter did not differ between treatments (Fig. 1e). The higher percentage of dead rosettes (Fig. 1f) suggested a higher turn-over rate: both rosette production and rosette mortality had very likely been higher in the nutrient added plots. This was due to both an increase in the proportion of rosettes that had flowered (2.3% vs. 19%) in the control and fertilized group respectively), and an increase in the proportion of rosettes that were dead but had not flowered (15% vs. 29%) respectively. On average 30% of the rosettes were connected by rhizomes to another rosette. The other connections had decayed over the three years of the study. This percentage increased significantly with the number of rosettes, but its variation was explained by neither the bud removal nor the nutrient treatment.

Site survey

The grasslands (with no top soil removal) ranged in mown biomass from 197 to 363 g m⁻² and had comparable vegetation height (Table 1). The areas in which the top soil was removed had lower biomass (64 and 98 g m⁻²), no humus layer and significantly more bare soil than grasslands. *Cirsium dissectum* rosette density varied greatly between plots which overruled possible differences between sites. The same is true for the percentage of rosettes that flowered per plot, which was highest in the most productive grassland and in one sod-cut area. Whether a rosette flowered or not was not correlated with rosette density or plot productivity in either vegetation type. Seedlings were only found in the sod-cut areas.

Average plant size as measured as the dry weight of the caudex did not differ between grasslands, but tended to be lower in the sod-cut areas. The caudex was heavier of flowering rosettes than of vegetative rosettes in both grasslands and sod-

cut areas (Table 2 and Fig. 2). The number of rhizomes produced by an excavated rosette was positively correlated with its caudex weight in all sites except for site B and N (Fig. 2), and was also positively correlated with plot productivity within the grasslands. Flowering rosettes, which had heavier caudices, produced more rhizomes than vegetative rosettes (Table 2; e.g. 0.32 vs. 2.0 in site B, 0.29 vs. 1.0 in site N, and 0.82 vs. 3.0 in site V, for respectively vegetative and flowering rosettes). Rhizome length and depth did not differ systematically between sites, but in the sod-cut area of site n only short shallow rhizomes were found and no long runners.

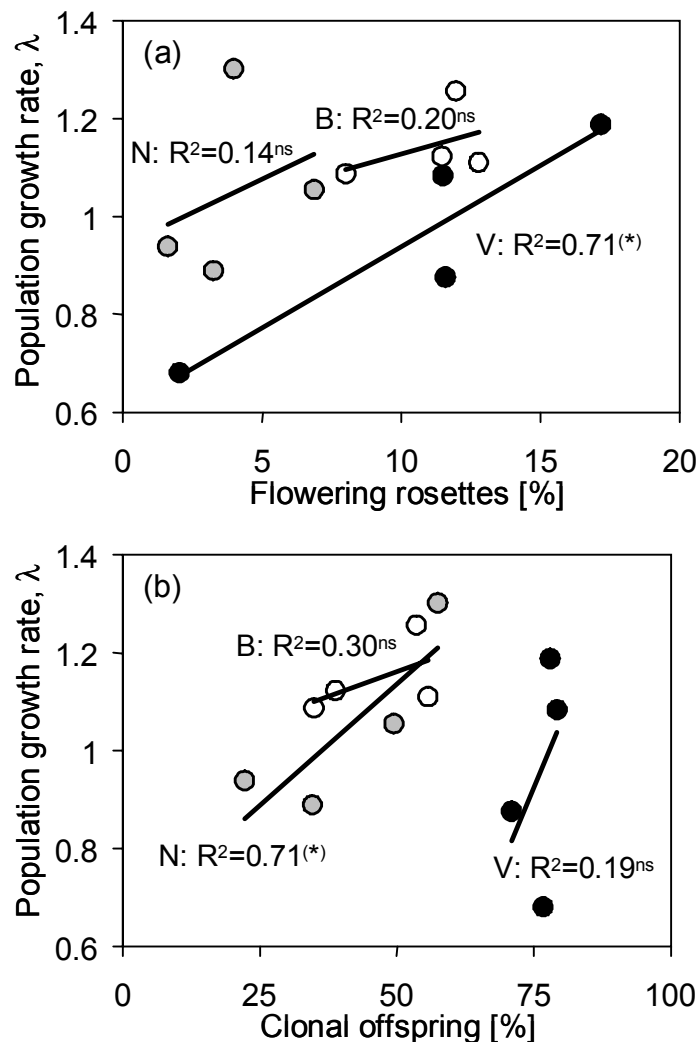


Figure 6.

Correlation between projected population growth rate, λ , and the percentage of all rosettes that are flowering (*flow* + *cl.f*) or are newly formed by clonal propagation (*cl.v* + *cl.f*) in the stable stage distribution. The sites are: Bennekomse meent (B; open circles), Konijnendijk (N; grey circles) and Veerslootlanden (V; black circles). Within each site correlations are tested with linear regression. ns = not significant; $(*) = p < 0.10$

Table 1.

Overview of the site survey. At each site five randomly chosen plots of 50x50 cm with at least five *Cirsium dissectum* rosettes (graslands) or two rosettes (sod cut areas) were described: mean vegetation height, vegetation cover, thickness humus layer and rosette density. Five rosettes per plot were randomly selected and carefully excavated. Connected rhizomes were followed to their destination. For each parameter different letters stand for significant site differences (Tukey's post-hoc test, $\alpha = 0.05$). In order to improve homogeneity of variance the vegetation height and rosette density data were ln-transformed before being tested. Sites were: Nijkampse heide (h and H), Stelkampsveld (S), Bennekomse meent (B), Konijnendijk (n and N) and Veerslootlanden (V).

	h	n	H	S	B	N	V
Plot parameters							
top soil removed	yes	yes	no	no	no	no	no
mown biomass [g m ⁻²]	64 a	98 ab	197 abc	263 bc	278 bc	315 c	363 c
vegetation height [cm]	16 ab	15 a	16 ab	32 b	28 ab	29 b	29 b
vegetation cover [%]	48 a	39 a	92 b	90 b	92 b	93 b	86 b
thickness humus layer [cm]	0 a	0 a	9 bc	10 bc	12 c	16 d	6 b
<i>C. dissectum</i> rosette density [No. m ⁻²]	84 ab	18 a	133 b	89 b	49 ab	120 b	42 ab
flowering % <i>C. dissectum</i>	20 a	8 a	6 a	9 a	6 a	7 a	15 a
seedling % <i>C. dissectum</i>	3 a	15 a	0 a	0 a	0 a	0 a	0 a
Selected <i>C. dissectum</i> rosette parameters							
dry weight caudex [mg]	36 ab	21 a	61 bc	84 c	67 bc	69 bc	62 bc
max. leaf length [cm]	8 ab	5 a	11 bc	14 cd	14 cd	15 d	17 d
No. rhizomes per rosette	0.43 ab	0.11 a	0.28 a	0.42 ab	0.46 ab	0.33 a	1.00 b
rhizome length [cm]	15 b	2 a	16 b	11 ab	8 ab	10 ab	17 b
rhizome depth [cm]	3 ab	1 a	4 b	3 b	3 b	2 ab	3 b

Rhizome formation experiment

Rosettes of *C. dissectum* from sites B and V had significantly lower biomass than those from site H but no differences were seen between the other sites (Fig. 3). The presence of the grass *Agrostis capillaris* reduced average biomass production by a factor 5.8 irrespective of site of origin. Variation in the number of rhizomes produced was explained fully by the grass treatment and total *C. dissectum* biomass, indicating that rosette production depends strongly on rosette size. No differences between sites were found in the number of rhizomes per unit dry weight.

Population dynamics

The data on survival, rosette growth and clonal propagation from the permanent plots in the meadows B, N and V resulted on average in projection matrices with growth rates around one (λ ranging from 0.68 to 1.30; Fig. 4). Although the variation in λ of the three sites overlapped, there were distinct differences between the populations: rosettes in site N had smaller leaves than rosettes in the other two sites, and site V had both a higher rate of clonal propagation and strikingly lower rosette survival rates (Table 3). The seed addition experiment resulted in very low seedling and small rosette

establishment rates (0.00025 and 0.00050 per seed; Chapter 4). Only three seedlings in total were found during the five summers in which the permanent plots were censused and no seedling survival was observed.

Due to such low fecundity, seedlings made up less than 0.1% of the projected stable population structure in all three populations, even while seedling survival was kept conservatively high. The elasticity diagrams confirmed that the rosette survival had higher contributions to λ in site B and N, but that clonal propagation by one-year old clonal offspring was most important in most productive site V (Fig. 5). The correlation between λ and the projected population structure differed between sites when the four years were compared: in site V population growth rate was positively correlated with the percentage of flowering plants, but the percentage of rosettes that were newly produced by clonal propagation was constant at about 75% over the years (Fig. 6). The opposite pattern was found in site N where there was only a weak correlation of λ with flowering percentage, but the percentage of clonal offspring was twice as high in good years, compared to bad years. Site B was intermediate to the other two.

Life Table Response Experiments

In order to analyze what differences in matrix elements could explain the variation in the projected population growth rate, λ , between sites and years, we decomposed the variation in λ according to the LTRE techniques. The model fitted the data well as the differences between the observed and modeled λ was on average 0.13%. The two-way LTRE revealed larger year than site effects, and interaction effects were almost as large as the year effects: the means of the absolute values of the site, year and interaction effect were 6.51, 11.18 and 10.22 respectively. The relatively low λ in site V as compared to the average reference matrix was mostly due to low survival of one year old clonal offspring (Fig. 5). However, this was to a large extent compensated by positive contributions to λ by higher growth rates (G) and especially higher clonal reproduction (C). The other two sites showed the reverse pattern of contributions.

Discussion

Explanations for increased sexual reproduction under nutrient enrichment

Higher biomass turn-over seems to be the most likely explanation for the increase in seed production in the three-year allocation experiment. The observation that *Cirsium dissectum* had spread further in nutrient-rich plots and that more dead rosettes were found in these plots strongly suggests that the biomass and clonal growth rate were higher, whereas the total biomass and number of living rosettes did not differ from the

Table 2.

Pearson correlation coefficients between combinations of parameters of randomly selected *Cirsium dissectum* rosettes and their plot characteristics, within two vegetation types: 123 rosettes in five grasslands, and 36 rosettes in two areas of which the top soil was removed. The plant parameters were the dry weight [g] of the caudex, the number of rhizomes made by the rosette, and its flowering status (0=no, 1=yes). The parameters of the 50x50 cm plots were: mown vegetation biomass [g m⁻²] and the density of *C. dissectum* rosettes [No. m⁻²].

<i>Grasslands</i>	Caudex [mg]	Flowering	No. rhizomes	Mown biomass
Flowering	0.27 **			
No. rhizomes	0.43 **	0.43 **		
Mown biomass [g m ²]	0.08	0.01	0.20 *	
Rosette density [No. m ⁻²]	-0.03	-0.10	-0.15	0.00
<i>Sod-cut areas</i>	Caudex [mg]	Flowering	No. rhizomes	Mown biomass
Flowering	0.50 **			
No. rhizomes	0.57 **	0.31 (*)		
Mown biomass [g m ²]	-0.37 *	-0.16	-0.22	
Rosette density [No. m ⁻²]	0.25	0.16	0.39 *	-0.32 (*)

control group at harvest. The latter would further suggest that the decay rate is also higher. Such increases in metabolism are also found when the effect of increased fertility on root decomposition was studied (van der Krift et al. 2001). In our case higher growth rates probably allow a greater proportion of rosettes to reach the size required for flowering. High growth rates also result in more clonal offspring, but their number seems to be balanced by higher rosette mortality.

The demographic field data seem to support this theory: the most productive site (V) had higher clonal growth rate and lower rosette survival. Of course some caution is needed as only three sites were compared, that tend to differ not only in productivity, but also in other ways. For example site V is peaty, whereas the others are more sandy. The patterns of the nutrient enrichment treatment and the site gradient are strikingly similar though. Site V also had the highest percentage of flowering rosettes in the projected population structure, although flowering was highly variable and depending on good years.

Other explanations for increased sexual reproduction are less convincing. Loehle (1987) hypothesized that when high nutrient availability alleviates the nitrogen limitation of seed production, sexual reproduction becomes less costly and should be favored. Reekie (1999) agrees that variation in the costs of modes of reproduction may alter trade-offs between sexual reproduction, clonal propagation, and plant growth. In his study on the rhizomatous grass *Agropyron repens* however, high nitrogen availability also lowers the costs of rhizome allocation, although the trade-off between both modes of reproduction was also influenced by light levels (Reekie 1991). The results of our site survey show that rosette biomass does not differ

between meadows, but that relative clonal allocation is highest in the most productive site V. This may indeed be due to lower costs of rhizome production, but may also be explained by higher growth rates when rhizomes are stronger sinks for nutrient allocation than rosettes. Our data are not suitable to make this distinction, and it could be that both explanations, higher biomass turn-over and lower costs of reproduction, are additive. However, it seems unlikely that nitrogen availability lowered the costs of seed production as the nutrient treatment did not interact with costs of seed production in the allocation experiment: the bud removal treatment revealed similar costs of sexual reproduction in the nutrient rich treatment as in the low nutrient treatment (Chapter 3).

Differences between sites in allocation to sexual reproduction may also have a genetic basis. Distinct *C. dissectum* populations in the Netherlands have been shown to differ significantly in AFLP-markers, although variation within sites was larger than between sites (Smulders et al. 2000). We found some significant differences in growth rates between individuals from different populations with a suggestion that more productive sites may have individuals with a lower growth rate. This may be due to the nutrient-poor, sandy soil of the cold greenhouse experiment, which is more similar to the sites with a low-productive vegetation, indicating a degree of adaptation to local conditions. However, it does not give an explanation for higher rosette growth rates in certain sites. Neither does the variation in rhizome production in the common environment experiment, which was not influenced by site of origin, but was fully explained by direct and indirect (through plant biomass) effects of the grass treatment. We conclude that genetic differences are unlikely to be the main explanation for the patterns of sexual and clonal reproductive allocation between populations. Disentangling the exact role of genetics in *C. dissectum* on its vital rates within and between populations requires more detailed research.

The population dynamics of Cirsium dissectum

The population dynamics of *C. dissectum* are almost entirely based on clonal propagation as survival and growth of seedlings is very rare. Kay and John (1994) found no seedlings at all in their survey of British populations. The three seedlings we found in five years made up 0.1% of the observed rosettes per census. This low number of seedlings in *C. dissectum* is caused partly by poor seed production but primarily by very low establishment rates within vegetation stands (Chapter 4). This low seedling recruitment rate could potentially lead to a reduction in genetic diversity. On the other hand, genets of clonal species can be very long-lived and require little seedling recruitment per year to compensate for genet mortality and to maintain genetic diversity (Eriksson 1989; Cain 1990; Watkinson & Powell 1993), and modest but repeated seedling recruitment can occur in clonal species (Suzuki et al. 1999). The effect of low seedling recruitment rates on the genetic diversity and the implications of this for the health of *C. dissectum* populations is an area for further research.

The elasticity and the LTRE analyses both point to clonal propagation and

Table 3.

Year-to-year population transition matrices with six classes: seedlings (*sdI*), small vegetative rosettes (*sml*), large vegetative rosettes (*lrg*), flowering rosettes (*flow*), vegetative clonal offspring (*cl.v*), and flowering clonal offspring (*cl.f*). Above: each existing element of the matrix is assigned to one of six life-history components: fecundity (*F*), growth (*G*), clonal reproduction (*C*), survival clonal offspring (*T*), stasis (*S*), and retrogression (*R*). Below: for each *Cirsium dissectum* population the means and coefficients of the spatiotemporal variation (CV) of each matrix element. Under each matrix the mean and CV of the mortality probability of each class are given. The sites are: Bennekomse meent (B), Konijnendijk (N) and Veerslootlanden (V).

	<i>sdI</i>	<i>sml</i>	<i>lrg</i>	<i>flow</i>	<i>cl.v</i>	<i>cl.f</i>
<i>sdI</i>	S ₁₁	-	-	F ₁₄	-	F ₁₆
<i>sml</i>	G ₂₁	S ₂₂	R ₂₃	F ₂₄	T ₂₅	F ₂₆
<i>lrg</i>	-	G ₃₂	S ₃₃	-	T ₃₅	-
<i>flow</i>	-	G ₄₂	G ₄₃	-	T ₄₅	-
<i>cl.v</i>	-	C ₅₂	C ₅₃	C ₅₄	C ₅₅	C ₅₆
<i>cl.f</i>	-	C ₆₂	C ₆₃	C ₆₄	C ₆₅	C ₆₆

Site B	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
<i>sdI</i>	0.300	0	-	-	-	-	0.002	29	-	-	0.002	29
<i>sml</i>	0.150	0	0.054	131	0.079	111	0.005	29	0.061	60	0.005	29
<i>lrg</i>	-	-	0.453	24	0.544	30	-	-	0.498	25	-	-
<i>flow</i>	-	-	0.018	116	0.110	44	-	-	0.108	17	-	-
<i>cl.v</i>	-	-	0.307	25	0.302	25	1.915	23	0.323	16	2.049	19
<i>cl.f</i>	-	-	0.008	200	0.019	81	0.138	90	0.027	40	0.129	7
dead	0.550	0	0.475	39	0.268	37	1.000	0	0.333	22	1.000	0

Site N	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
<i>sdI</i>	0.300	0	-	-	-	-	0.007	30	-	-	0.007	30
<i>sml</i>	0.150	0	0.482	32	0.277	37	0.014	30	0.426	12	0.014	30
<i>lrg</i>	-	-	0.110	76	0.278	61	-	-	0.150	54	-	-
<i>flow</i>	-	-	0.040	54	0.063	102	-	-	0.033	46	-	-
<i>cl.v</i>	-	-	0.396	54	0.406	54	1.352	55	0.382	56	2.100	10
<i>cl.f</i>	-	-	0.014	147	0.012	200	0.038	200	0.002	129	0.000	0
dead	0.550	0	0.368	29	0.382	27	1.000	0	0.391	15	1.000	0

Site V	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
<i>sdI</i>	0.300	0	-	-	-	-	0.003	9	-	-	0.003	9
<i>sml</i>	0.150	0	0.062	96	0.071	84	0.006	9	0.020	83	0.006	9
<i>lrg</i>	-	-	0.611	11	0.124	102	-	-	0.173	30	-	-
<i>flow</i>	-	-	0.000	0	0.145	128	-	-	0.029	79	-	-
<i>cl.v</i>	-	-	0.549	22	0.538	27	2.207	16	0.477	21	1.969	9
<i>cl.f</i>	-	-	0.124	96	0.077	132	0.365	100	0.035	45	0.147	41
dead	0.550	0	0.327	39	0.660	41	1.000	0	0.778	7	1.000	0

survival of clonal offspring as the most important life-history components. The LTRE results suggest that rosette survival trades off with clonal propagation, whereas rosette growth is positively correlated with clonal propagation. Although the percentage of flowering rosettes is highly variable between years, sexual reproduction plays no role in the LTRE. But flowering rosettes do have a higher rate of clonal propagation, although it is not restricted to flowering plants, as e.g. in *Veratrum album* (Kleijn & Steinger 2002).

Rhizome length is highly variable (from close to the parent plant up to 40 cm in our study) and does not differ significantly between populations. Neither was it affected by increased nutrient levels, which is consistent with other studies (de Kroon & Knops 1990; Evans 1992; Dong & de Kroon 1994; Dong & Alaten 1999). The thick rhizomes of *C. dissectum* seldom deviate from a negative hyperbolic trajectory through the soil and seem not to be hampered by roots or tussocks of other species or soil types (E. Jongejans, personal observation).

Implications for the conservation of Cirsium dissectum

Cirsium dissectum is restricted to a limited abiotic range due to its sensitivity to effects of acidification (de Graaf et al. 1998; Lucassen et al. 2003). We add that *C. dissectum* is also a poor below-ground competitor and that within the range of abiotic conditions it occurs, nutrient availability has a profound effect on the population dynamics of this endangered species. High productivity increases rosette turn-over, which in itself does not necessarily decrease population size, but it is expected to decrease genetic diversity as the probability of genet mortality increases. This increase in genet mortality is not balanced by sexual recruitment from seed. In fact, the increased biomass of competitive species such as *Molinia caerulea* under conditions with increased nutrients may further decrease seedling recruitment due to a reduction in gaps for establishment.

Drainage and nitrogen deposition are likely to have a further negative impact as this species is unable to accumulate biomass. This inability and its short stature (Hegde & Ellstrand 1999) make *C. dissectum* a poor competitor, explaining its restriction to nutrient-poor, wet habitats (Grime 1979; Tilman 1987). The role of rhizomes in this clonal species seems to be restricted to spreading new rosettes, as the rhizomes are relatively short-lived and do not function as storage organ or as a means of resource sharing between rosettes after the initial phase.

Nature management aimed at conserving populations of this endangered species should focus on restoring and maintaining hydrologically suitable conditions, and should counteract succession and nutrient enrichment through mowing and hay removal. Sod-cutting seems necessary for seedling establishment. Negative effects of initial ammonium peaks after sod-cutting (Dorland et al. 2003) do not seem to limit seedling establishment in the long run, provided that seed sources are nearby. Increased seed production in nutrient enriched grasslands can be expected to be helpful near such favorable establishment conditions.

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A hierarchical population matrix analysis of the effects of nutrient enrichment: a synthesizing discussion

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Summary

Nutrient enrichment decreases species richness in grasslands through accelerated secondary succession. A previous experiment on four grassland herbs showed that species with different life history strategies are differently affected in their survival rate, plant size and sexual reproductive allocation. In this paper we investigate the contrasting effects of fertilization: do negative effects on survival and recruitment outweigh positive effects on plant size and seed production? This was tested with a hierarchical population matrix model in which matrix elements were functions of life history components, which in turn were functions of the plant traits size, flowering threshold size and relative sexual reproductive allocation. The model results differed for species with short-lived and long-lived rosettes. For the former species (*Hypochaeris radicata* and *Cirsium dissectum*) high mortality rates overruled other effects and caused strong population declines, whereas in the long-lived species, in the absence of increased mortality, decreased recruitment had little effect on population growth rate (*Centaurea jacea*) or was compensated by increased flowering and seed production (*Succisa pratensis*). But also the long-lived species will face strong population declines when eutrophication lasts for more than a few years and even these rosettes will experience high mortality rates.

Keywords: *biomass allocation*, *Centaurea jacea*, *Cirsium dissectum*, *elasticity*, *Hypochaeris radicata*, *life history components*, *LTRE*, *nutrient enrichment*, *plant trait*, *sensitivity*, *sexual reproductive allocation*, *Succisa pratensis*, *succession*

Introduction

Herbaceous perennials in grasslands are outcompeted by grasses and more competitive herbs when the vegetation biomass increases due to natural succession. Human induced nutrient enrichment accelerates this process significantly. Eutrophication has already caused declines in species richness in many grasslands

(Neitzke 2001; Stevens et al. 2004). Many of the declining species are now endangered and targets of nature conservation and restoration efforts. These efforts however may gain in effectiveness by a better understanding of how exactly components of the life cycle of these herbs are affected by nutrient enrichment and how population growth rates are reduced.

The effects of nutrient enrichment on a plant population depends strongly on how individual plants respond to the changing growing conditions. Plants of nutrient-poor grasslands that are able to enhance their competitive ability when nutrient limitation is reduced by fertilization, will form more stable populations than plants that can not adapt. Contrastingly, plants may also be expected to increase their seed production in order to increase the capacity to colonize more favourable sites. The four grassland herbs that are studied in this paper have been shown to be rather flexible in their life-history decisions (Chapters 2 and 3). Not only were these perennial herbs able to compensate for lost flower buds (short-lived species faster than longer-lived species) and for lost rosette buds (clonal species faster than more rarely ramifying species), but they were also able to switch from sexual reproduction to clonal propagation when flowering was inhibited. Furthermore, a three-year garden experiment revealed that plants respond to nutrient enrichment in different ways (Chapter 3). In that experiment we mimicked nutrient enrichment during secondary succession by fertilizing plots with these herbs and competing grass tussocks. The main results of the fertilization treatment were that the surviving, fertilized plants were larger than the plants in the unfertilized plots and that they increased their allocation to seed production. Notwithstanding the demonstrated costs of seed production, total biomass of surviving plants was increased, while the allocation to storage increased rather than decreased as would be expected. Thus both persistence and escape mechanisms are enhanced by nutrient enrichment.

Plant mortality increased due to nutrient enrichment as well in the two species with short-lived rosettes (Chapter 3). Vegetation biomass also strongly influences the recruitment rates as some species depend on bare soil for successful seedling establishment, which is an important aspect of the sexual reproduction pathway (Chapter 4). Especially the two mainly clonally reproducing herb species established more seedlings in sod-cut areas than in grasslands. Also differences in the standing crop of grasslands matter for recruitment: Soons et al. (2003) found that the establishment rates of the two mainly clonally reproducing species were further reduced to zero at higher-productivity meadows as compared with nutrient-poor meadows. The recruitment of the two more sexually reproducing species was less affected by increasing vegetation biomass. Thus nutrient enrichment can lead to decreased survival and reduced seedling recruitment through increased above-ground competition depending on the species studied. Whereas reduced survival and seedling establishment can cause population declines, the enhanced sexual reproduction reported in chapter 3 can be expected to increase population growth rate. However, when investigating the effects of nutrient enrichment on the population dynamics it is not possible to evaluate one aspect of the life cycle without considering all other parts in concert (Franco and Silvertown 2004).

To investigate the impact of succession, populations of the same species in different successional stages (Oostermeijer et al. 1996) or of species characteristic for different successional stages (Silvertown et al. 1993) have been compared. The general pattern that emerges from these studies is that the relative importance of sexual reproduction declines in time with secondary succession. Furthermore, strong reductions in population size increase the risk of inbreeding. Inbred seeds in the long-lived perennial *Succisa pratensis* have lower establishment rates and inbred seedlings lower growth rates, which reduces the population growth rate and population size even more: the population becomes trapped in an extinction vortex (Chapter 6). Both inbreeding depression and site productivity also reduce the colonization capacity of the same grassland herbs (Soons & Heil 2002) and inbreeding depression and low habitat quality have been shown to interact and reduce several fitness parameters (Vergeer et al. 2003).

A closer examination of the population dynamics in the field revealed that the species studied can respond very differently to the same spatial and temporal variation in growing conditions. In the long-lived *Succisa pratensis* some populations had lower population growth rates because the rosettes grew slower, or because seed set was prohibited by early mowing. Whereas increases in other life history components partly compensated these negative effects on the population growth, all components were lower in the site with the highest vegetation biomass (Chapter 5). The rhizomatous *Cirsium dissectum*, which has short-lived rosettes, showed a contrasting response to the same productive site: the clonal propagation rate was higher than in two other sites, but rosette mortality was also higher (Chapter 7). These species therefore displayed two different responses to nutrient enrichment: the *S. pratensis* population became more senile (sensu Oostermeijer et al. 1996: consisting of adult plants almost without seedling recruitment and with a low population growth rate), while the *C. dissectum* population became more dynamic than the species-average population dynamics: it had a higher rosette turn-over and the temporal variation in population growth rate was high. Whether these field patterns between populations over a gradient of nutrient richness are the result of the responses of the plant individuals as observed in our experiments with nutrient enrichment remains untested.

In this paper we aim to investigate how positive direct effects of nutrient enrichment on plant traits and negative effects that work indirectly through increased competition with neighboring plants together affect population growth rate. To that aim we construct a hierarchical matrix model that allows for the evaluation of the importance of life history components and plant traits on the population growth rate (cf. van Tienderen 2000). We therefore combine demographic data from a nutrient-poor site (Chapters 5 and 7) and the results of the nutrient richness experiment (Chapter 3). In this modeling exercise we firstly test how the population growth rate is affected by nutrient enrichment and whether the population changes into a 'senile' population (lower proportion of seedlings), or into a more dynamic population (higher proportion of seedlings and clonal offspring). Secondly we study how the relative importance of the life history components and plant traits change when nutrient enrichment is simulated

in natural populations. We expect plant size and traits of sexual reproductive allocation to become more important for the population growth rate in fertilized populations, and seedling recruitment to become less important for local population dynamics. Thirdly, we expect short-lived herb species to suffer more from the negative indirect effects of nutrient enrichment than longer-lived species.

Materials and methods

Study species

The four study species were selected for their contrasting life time expectancy, clonality and sexual reproduction rates, in order to cover a range of possible life history strategies in grassland herbs. The year-to-year survival of large, non-flowering rosettes was highest in *Succisa pratensis* and lowest in *Cirsium dissectum* (Chapters 5 and 7). The last species however had the highest number of clonal offspring per flowering rosette. *Centaurea jacea* was intermediate in both survival and clonality. *Hypochaeris radicata* was the most short-lived perennial with little ramification and relatively low adult survival (although higher than in more productive habitats (de Kroon et al. 1987)). Sexual reproduction is negatively correlated with clonality: the clonal *C. dissectum* and *C. jacea* had much lower fecundity than the only rarely ramifying *S. pratensis* and *H. radicata*.

For this model study we used the combined data of four year-to-year population transitions for each species. These demographic data were collected for all species in the same permanent plots in the Dutch nature reserve Konijnendijk (52°02' N, 6°26' E) from 1999 to 2003 (Chapters 5 and 7).

Population matrix construction

We used stage-based matrices (Lefkovitch 1965) to model the population dynamics of these herb species. Because the aim of this study was to explicitly investigate the role of plant size, we had to reconstruct the reported 6x6 matrices (Chapters 5 and 7) as these consisted of a stage classification which was partly based on plant size. In this paper the 5x5 stage classification is not based on plant size, but entirely based on sexual reproduction, flowering, survival and clonal propagation (Fig. 1b): seedlings or new side rosettes can become adults after one year or die, and adults and side rosettes can either flower or stay vegetative.

In order to be able to investigate the importance of lower-level parameters we formulated a hierarchical pathway (Fig. 1a) in which matrix elements were functions of life history components, and these life history components were functions of plant traits (van Tienderen 2000). The life history components were seed production,

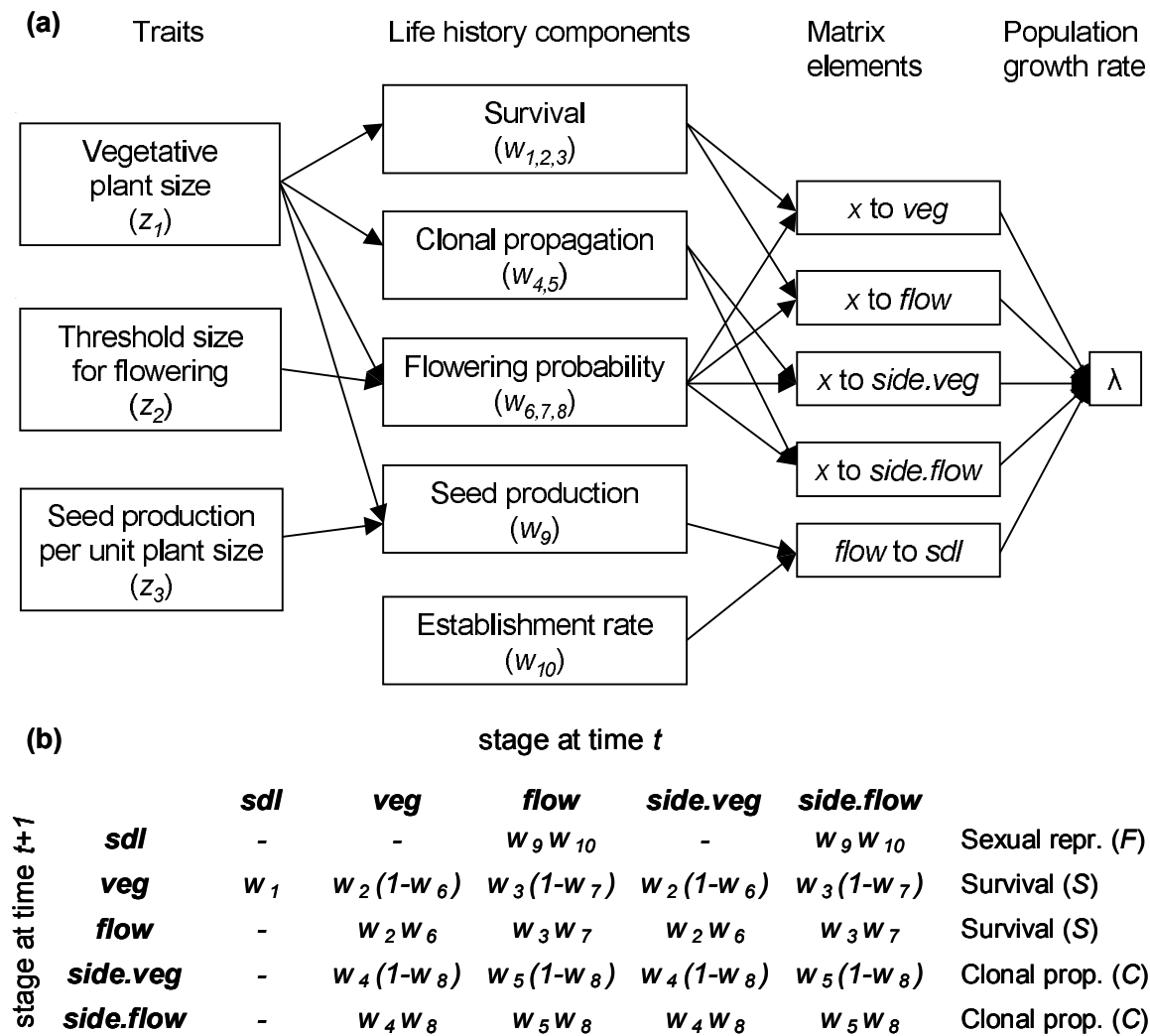


Figure 1.

(a) Path diagram of the contributions of plant traits to consecutively life history components, matrix elements and population growth rate. The plant traits are assumed to be independent.

(b) Construction of a 5x5 population transition matrix with 10 life history components. The five stage classes are (the x in the upper diagram aggregates all stage classes):

sdl = new seedlings,
veg = non-flowering rosettes older than one year,
flow = flowering rosettes older than one year,
side.veg = new, non-flowering clonal offspring,
side.flow = new, flowering clonal offspring.

The 10 life history components are:

w_1 = survival of *sdl*,
 w_2 = survival of *veg* and *side.veg*,
 w_3 = survival of *flow* and *side.flow*,
 w_4 = clonal propagation by *veg* and *side.veg*,
 w_5 = clonal propagation by *flow* and *side.flow*,
 w_6 = flowering probability of surviving *veg* and *side.veg*,
 w_7 = flowering probability of surviving *flow* and *side.flow*,
 w_8 = flowering probability of new *side.veg* and *side.flow*,
 w_9 = seed production per *flow* and *side.flow*,
 w_{10} = *sdl* establishment rate per seed.

The elements of the first row of the matrix represent sexual reproduction (F), the elements of the 2nd and 3rd row are rosette survival (S) chances and the bottom two rows represent clonal propagation (C).

seedling establishment, and the survival, flowering probability and clonal propagation of different classes (Fig. 1b). However, the fate of non-flowering new side rosettes (*side.veg*) was considered to be identical to that of non-flowering adults (*veg*), as was the fate of flowering new side rosettes (*side.flow*) and flowering adult rosettes (*flow*). This was because the main differences in fate were observed between flowering and non-flowering rosettes, and because not in all species enough side rosettes were present for accurate quantification of their fate. For the same reason no distinction was made between the sexual reproduction of seedlings (*sdl*) by either *flow* or *side.flow*, and was assumed that all new side rosettes had the same probability of flowering in the same year they were produced. In total we defined 10 different life history components (Fig. 1b)

The next step was to quantify these 10 life history components, w_k , with the demographic field data by performing regression models with plant size, z_1 , as explaining factor. For seed production and clonal propagation we used linear regressions of the form:

$$w_k^N = a_k^N \cdot z_1 + b_k^N \quad (1)$$

in which a_k and b_k are constants and in which vegetative plant size was estimated by rosette size (the number of leaves times the maximum leaf length). The N -indices indicate natural population parameters. For the boolean life history components survival and flowering we used binary logistic regressions (Childs et al. 2004):

$$w_k^N = \frac{\exp(a_k^N \cdot z_1 + b_k^N)}{1 + \exp(a_k^N \cdot z_1 + b_k^N)} \quad (2)$$

in which a_k and b_k are the constants that shape the logistic regression. Seedling establishment, w_{10} , and seedling survival, w_1 , were derived from a seed addition experiment (Chapters 5 and 7) and were not constructed as functions of plant size but as constants. In *C. dissectum* w_5 , w_8 and w_9 were also constants because this species forms only one flower head per flowering rosette and because the data set on clonal propagation by flowering rosettes and the consecutive flowering probability of the new clonal offspring was too small to allow for regression analyses.

Besides the trait plant size, z_1 , two other traits were defined as well. These two traits shape the relationship between the amount of sexual reproduction and vegetative plant size. The threshold size for flowering, z_2 , was not estimated in the logistic regression analysis of the demographic data. But since the flowering threshold was sometimes affected by experimental nutrient enrichment, we defined z_2 as a reduction of plant size in the flowering probability function and with $z_2 = 0$ as default value for the natural population dynamics. The last plant trait, seed production per unit plant size, z_3 , determined the slope of the sexual reproduction-vegetative plant size relationship. This trait is equal by definition to a_9 parameter in the linear function for seed production.

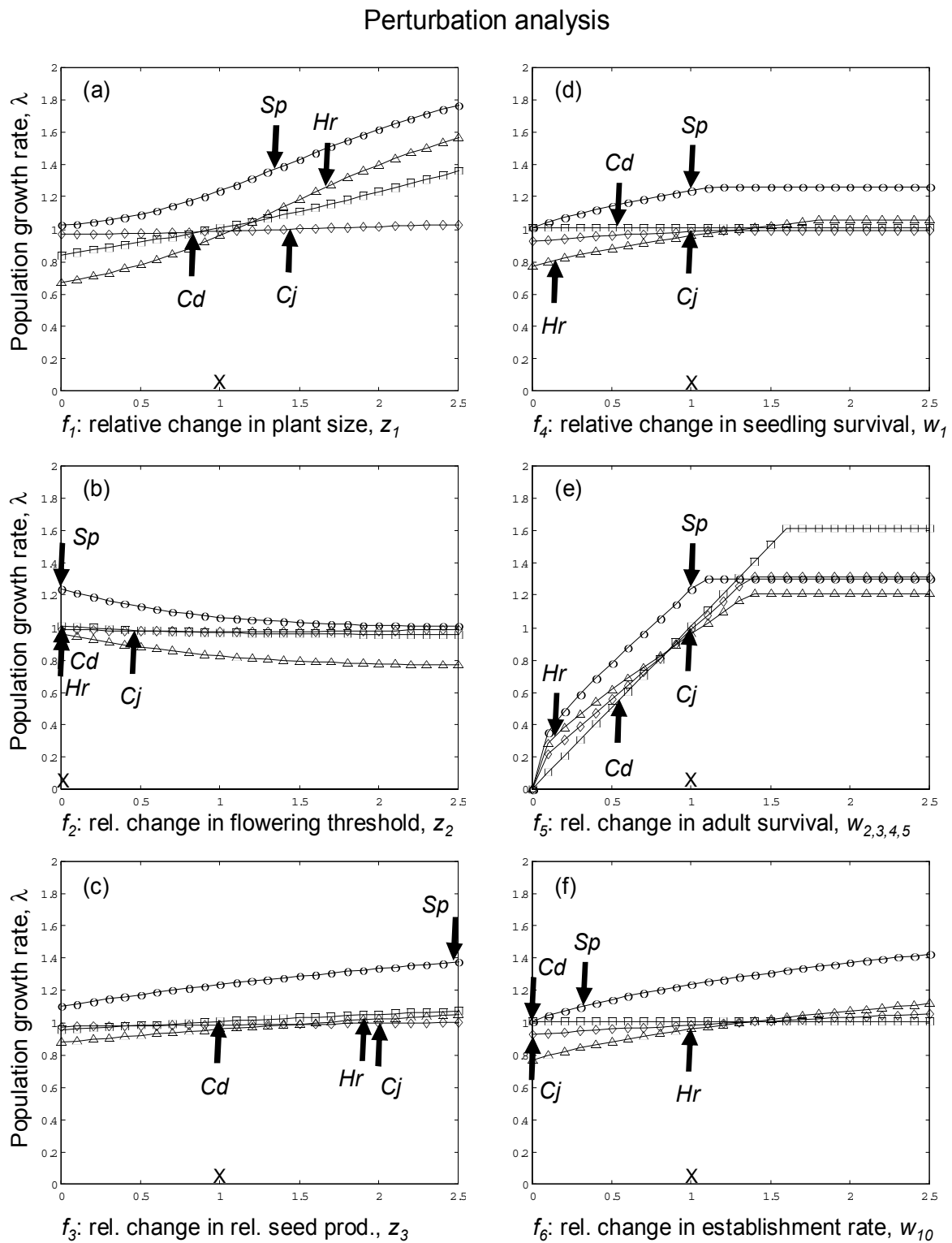


Figure 2.

Perturbation analysis on the impact of the six nutrient enrichment factors, f_i , on the population growth rate. In separate simulations one of the six f_i were varied from 0 to 2.5 while the other five f -values were kept constant at the default level of the natural populations (the values of f_i in the natural population dynamics are indicated on the x-axis with an 'x'). The four species are: *Hypochaeris radicata* (Hr, triangles), *Cirsium dissectum* (Cd, squares), *Succisa pratensis* (Sp, circles) and *Centaurea jacea* (Cj, diamonds). The arrows indicate the species-specific f -values as found in experiments on nutrient enrichment (see Table 2 for the exact values).

Effects of nutrient enrichment

Six different effects of nutrient enrichment on the population dynamics were considered: three on underlying plant traits and three on life history components directly. These effects were determined in a previous study in which we investigated the effect of nutrient enrichment on the survival, size and allocation patterns of these four herb species while they grew amongst dominating grasses in an experimental garden (Chapter 3). In order to translate the results of this experiment to the field situation, relative changes in plant traits and life cycle components were calculated from the experiment and then implemented in the matrix models (Table 1). The six factors, f_i , by which nutrient enrichment affected traits and components, were:

f_1 on plant size (z_1). In the experiment vegetative plant size was estimated by the total vegetative biomass (roots, leaves and stems). The effect on plant size was determined in the plants that survived until the end of the three-year experiment and estimated by the relative increase in vegetative biomass in the nutrient enrichment treatment compared to the no fertilization treatment. The functions of the life history components in which plant size was a significant parameter in the regressions on the field data, were modified by including the factor f_1 ; for instance in the linear function of w_k :

$$w_k^F = a_k^N \cdot (z_1^N \cdot f_1) + b_k^N \quad (3)$$

in which the N -parameters are the values in the regressions in the natural population dynamics (eq. 1), and the F -parameters are the new values in the fertilized matrix model.

f_2 on the threshold size for flowering (z_2). The threshold size for flowering was determined by the intercept of linear regressions of sexual reproductive biomass on vegetative plant biomass, in the fertilized and untreated groups separately. The value of f_2 was then estimated by the increase in a relative measure of z_2 : the ratio of the intercept in the regression and the mean vegetative biomass. Since nutrient addition affected z_2 only in *C. jacea*, z_2 was not modeled in the other species. For *C. jacea* the flowering probability functions w_6 and w_8 were modified by setting z_2 to a fraction f_2 of z_1 (f_2 was zero in all other cases):

$$w_k^F = \frac{\exp(a_k^N \cdot (z_1^N - z_2^F) + b_k^N)}{1 + \exp(a_k^N \cdot (z_1^N - z_2^F) + b_k^N)} = \frac{\exp(a_k^N \cdot (z_1^N - z_1^N \cdot f_2) + b_k^N)}{1 + \exp(a_k^N \cdot (z_1^N - z_1^N \cdot f_2) + b_k^N)} \quad (4)$$

Only the factor f_2 is modeled here, while the other factors of nutrient enrichment are not taken into account in equation 4.

f_3 on seed production per unit plant size (z_3). The slope of the regression of sexual reproductive biomass on vegetative biomass changed significantly in three species (but not in *C. dissectum*). In these three species the function of seed production, w_9 , was modified as follows:

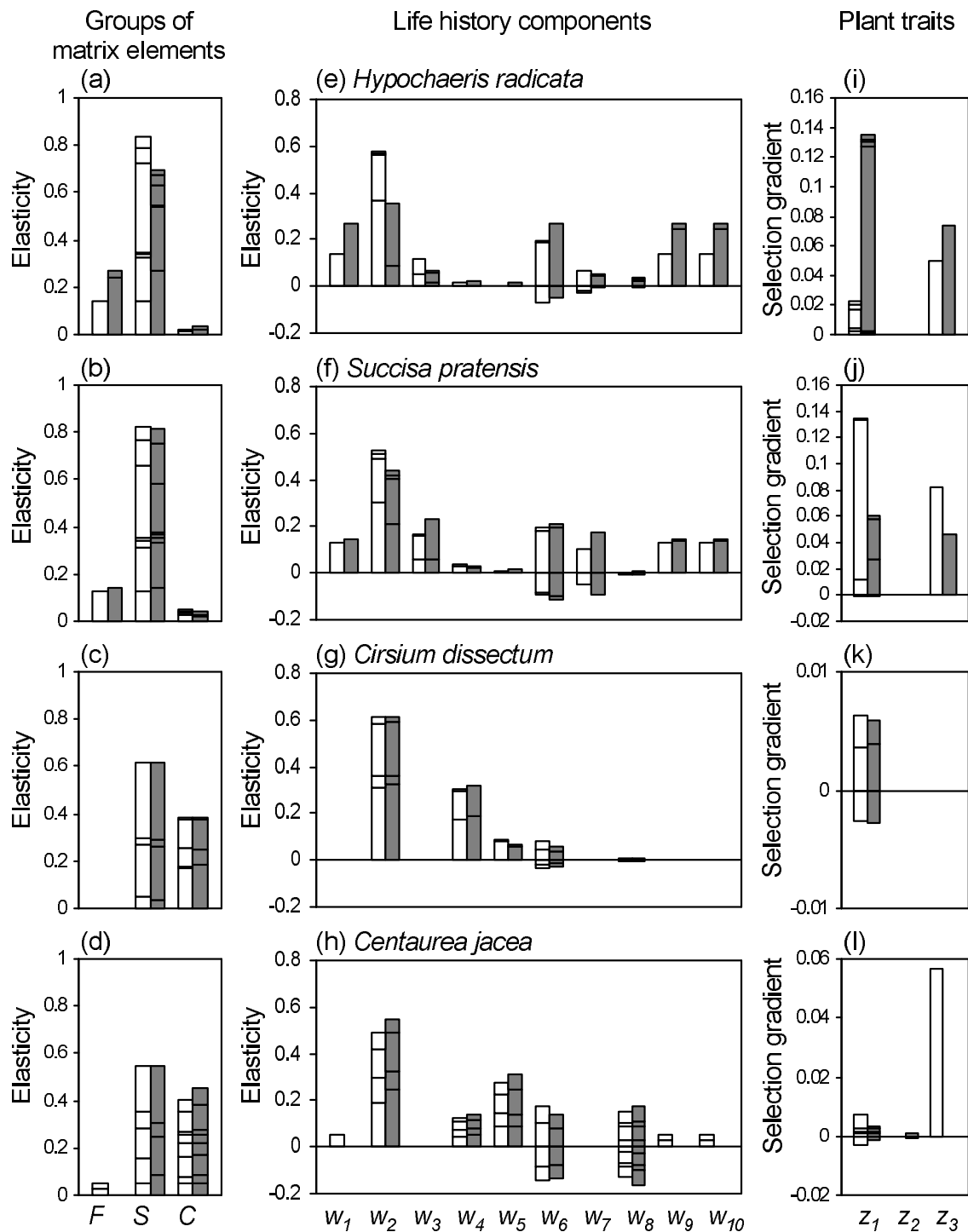


Figure 3.

Left two columns: elasticity of the population growth rate, λ , to groups of matrix elements (a/d; F = sexual reproduction, S = survival, C = clonal propagation), and to life history components (e/h; see Fig. 1 for the definitions of the w_k).

Right column: phenotypic selection gradient, β , of the plant traits (i/l; z_1 = plant size, z_2 = flowering threshold size, z_3 = sexual reproductive allocation). The white bars represent the natural population dynamics and the grey bars the fertilized population dynamics. The segmentation of the bars indicates the summation of the constituent parts of a certain parameter: elasticity values of matrix elements (a/d), elasticity values of life history components in single matrix elements (e/h) and selection gradients of traits in single life history components. Please note that the elasticity values of the lower-level parameters, w_k and z_r , do not sum to one like the elasticity values of the matrix elements, and that the selection gradient of a trait is equal to the elasticity value of trait divided by the trait mean.

$$w_9^F = (a_9^N \bullet f_3) \bullet z_1^N + b_9^N \quad (5)$$

Again we only entered the factor f_2 in this equation to illustrate its effect on the seed production function.

f_4 on seedling survival (w_1). The effect of nutrient enrichment on the survival of one-year old seedlings was not investigated separately. Therefore we assumed that the seedling survival rate was affected in the same way as the adult survival rate.

f_5 on adult survival (w_2 , w_3 , w_4 and w_5). The ratio of the number of surviving plants after three years in the nutrient enrichment treatment compared to the number of surviving plants in the unfertilized group was used as an estimate of the effect of extra nutrients on adult survival and clonal propagation. We assumed that besides rosette survival, clonal propagation by these rosettes was affected by the same factor because adult survival underlies those life history components. The mortality factor was modeled by multiplying the average value of a life history component with f_5 :

$$w_k^F = \bar{w}_k^N \bullet f_5 \quad (6)$$

f_6 on seedling establishment (w_{10}). The effect of high productivity on seedling establishment was investigated in a seed addition experiment (Soons et al. 2003). Here we use the ratio of the establishment rate in high productive sites (habitat class 2) compared to the establishment rate in low productive sites (habitat class 1) as an estimate of the effect of nutrient enrichment.

Elasticity analysis

To investigate the importance of all parts of the hierarchical path diagram (Fig. 1a) for the end product, population growth rate, λ , we calculated the absolute sensitivity, s , and relative sensitivity or elasticity, e , of λ to every part of the path diagram. Sensitivity measures how λ changes when a certain parameter is changed by a small amount while the other parameters are not changed. Elasticities however measure the effect on λ when a parameter is changed by a small factor. Therefore elasticities are a direct function of the mean of the parameter (Morris and Doak 2004). The sensitivity and elasticity of λ to matrix element a_{ij} is (de Kroon et al. 1986; Caswell 2001):

$$s_{a_{ij}} = \frac{\partial \lambda}{\partial a_{ij}} \quad (7)$$

$$e_{a_{ij}} = \frac{a_{ij}}{\lambda} \frac{\partial \lambda}{\partial a_{ij}} \quad (8)$$

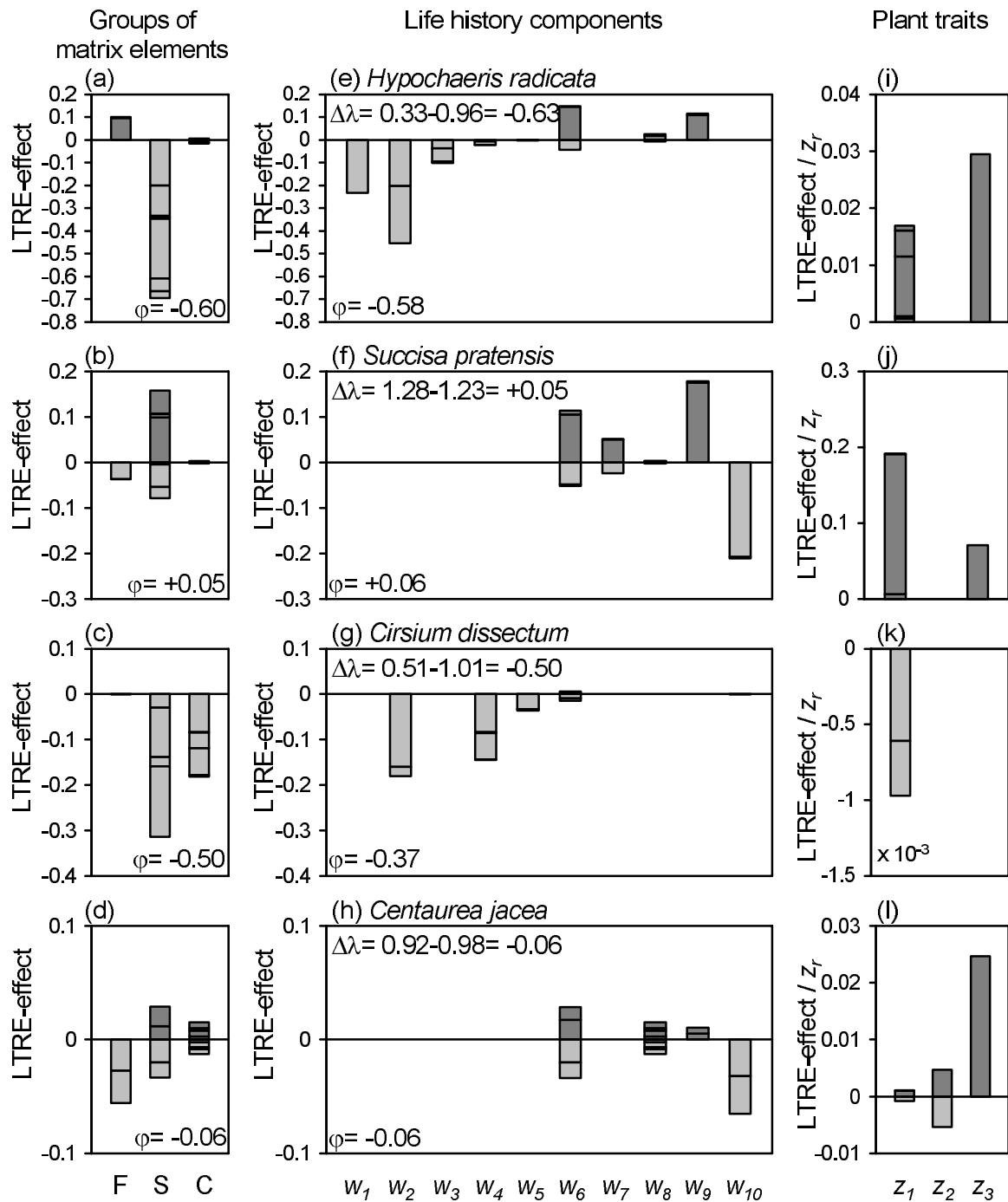


Figure 4.

Left two columns: Decomposition of the difference in population growth rate, λ , between the fertilized matrix and the natural population matrix that was used as the reference matrix. The difference in λ was decomposed into contributions by the variation in either matrix elements (a/d; F = sexual reproduction, S = survival, C = clonal propagation) or life history components (e/h; see Fig. 1 for their meaning) using an LTRE for each diagram separately. The segmentation of the bars indicates the summation of the components of each LTRE effect. In each diagram the overall fertilization effect, ϕ , is given, which can be compared to the deviation in λ of the fertilized population dynamics compared to the natural population dynamics.

Right column: Because the ϕ -values of the LTREs that decomposed variation in λ to variation in plant traits, z_r , deviated much from $\Delta\lambda$ (see the discussion section for an explanation) we divided the LTRE-effect of traits by the trait mean to rescale the effect on the variation in λ by the magnitude of the trait values (i,j,k,l; cf. Fig. 3i,j,k,l). As the trait mean we used the mean of the trait values in the natural and fertilized model. Please note that the scale of the y-axis in (k) ranges from 0 to - 0.0015.

Table 1.

Construction of life history components, w_k (Fig. 1), with regression models using rosette size (maximum leaf length times the number of leaves) as explaining factor. Survival ($w_{2,3}$) and flowering ($w_{6,7,8}$) probabilities were fitted with a binary logistic regression model (eq. 2). Clonal propagation ($w_{4,5}$) and seed production (w_9) were fit with a linear regression model (eq. 1). The values and significance of the regression parameters and the average w_k value of the field population are given to the right. The relative impact of fertilization, f_i , on the life history components and the plant traits is indicated behind the parameters they change. Plant traits are only considered to be affected when $p_a < 0.05$.

w_k	Regression	Rosette size		Regression parameters					\bar{w}_k	
		n	mean	a	b	p_a	p_b	R^2		
<i>Hypochaeris radicata</i>										
w_1	constant									0.539 $\cdot f_4$
w_2	bin. logistic	465	31.1 $\cdot f_1$	0.015	0.531	0.012	0.009	0.022		0.733 $\cdot f_5$
w_3	bin. logistic	89	46.6 $\cdot f_1$	0.058	-1.599	0.001	0.032	0.218		0.753 $\cdot f_5$
w_4	linear	465	31.1	0.001	0.005	0.086	0.774	0.006		0.028 $\cdot f_5$
w_5	linear	89	46.6 $\cdot f_1$	0.002	-0.072	0.023	0.147	0.058		0.034 $\cdot f_5$
w_6	bin. logistic	339	32.5 $\cdot f_1$	0.053	-3.412	0.000	0.000	0.229		0.158
w_7	bin. logistic	63	51.2	0.020	-1.789	0.180	0.030	0.040		0.313
w_8	bin. logistic	17	48.8 $\cdot f_1$	0.124	-8.730	0.035	0.025	0.608		0.065
w_9	linear	89	46.6 $\cdot f_1$	1.490 $\cdot f_3$	63.67	0.003	0.010	0.100		133.1
w_{10}	constant									0.019 $\cdot f_6$
<i>Succisa pratensis</i>										
w_1	constant									0.858 $\cdot f_4$
w_2	bin. logistic	750	35.2	0.008	2.267	0.345	0.000	0.003		0.929 $\cdot f_5$
w_3	bin. logistic	208	44.9	-0.029	4.084	0.105	0.000	0.033		0.942 $\cdot f_5$
w_4	linear	750	35.2	0.000	0.082	0.697	0.001	0.000		0.073 $\cdot f_5$
w_5	linear	208	44.9	0.000	0.085	0.921	0.197	0.000		0.091 $\cdot f_5$
w_6	bin. logistic	696	35.4 $\cdot f_1$	0.047	-2.961	0.000	0.000	0.156		0.216
w_7	bin. logistic	195	44.5 $\cdot f_1$	0.042	-2.073	0.000	0.000	0.107		0.448
w_8	bin. logistic	74	37.1 $\cdot f_1$	0.041	-4.423	0.048	0.000	0.128		0.051
w_9	linear	208	44.9 $\cdot f_1$	1.099 $\cdot f_3$	21.73	0.015	0.310	0.028		71.08
w_{10}	constant									0.023 $\cdot f_6$
<i>Cirsium dissectum</i>										
w_1	constant									0.450 $\cdot f_4$
w_2	bin. logistic	825	32.8 $\cdot f_1$	0.016	-0.012	0.003	0.950	0.015		0.625 $\cdot f_5$
w_3	constant									0.000 $\cdot f_5$
w_4	linear	22	28.9	-0.003	0.411	0.612	0.033	0.012		0.328 $\cdot f_5$
w_5	constant									2.481 $\cdot f_5$
w_6	bin. logistic	514	33.9 $\cdot f_1$	0.045	-4.369	0.000	0.000	0.081		0.056
w_7	constant									0.000
w_8	constant									0.006
w_9	constant									31.88
w_{10}	constant									0.001 $\cdot f_6$
<i>Centaurea jacea</i>										
w_1	constant									0.897 $\cdot f_4$
w_2	bin. logistic	350	41.3	0.009	0.641	0.147	0.015	0.009		0.730 $\cdot f_5$
w_3	constant									0.000 $\cdot f_5$
w_4	linear	350	41.3	0.001	0.158	0.575	0.005	0.001		0.186 $\cdot f_5$
w_5	linear	136	31.1	0.003	0.864	0.072	0.000	0.024		0.956 $\cdot f_5$
w_6	bin. logistic	255	42.3 $\cdot f_1$	0.029	-2.064	0.000	0.000	0.112		0.306
w_7	constant									0.000
w_8	bin. logistic	195	39.3 $\cdot f_1$	-0.015	-0.087	0.002	0.689	0.081		0.334
w_9	linear	136	31.1 $\cdot f_1 \cdot (1-f_2)$	0.186 $\cdot f_3$	22.88	0.000	0.000	0.195		28.669
w_{10}	constant									0.008 $\cdot f_6$

The sensitivity and elasticity of λ to life history component w_k is (Caswell 2001; Franco & Silvertown 2004):

$$s_{w_k} = \frac{\partial \lambda}{\partial w_k} = \sum_{ij} \frac{\partial \lambda}{\partial a_{ij}} \frac{\partial a_{ij}}{\partial w_k} = \sum_{ij} s_{a_{ij}} \frac{\partial a_{ij}}{\partial w_k} \quad (9)$$

$$e_{w_k} = \frac{w_k}{\lambda} \frac{\partial \lambda}{\partial w_k} = \frac{w_k}{\lambda} \sum_{ij} s_{a_{ij}} \frac{\partial a_{ij}}{\partial w_k} \quad (10)$$

And consequently, the sensitivity and elasticity of λ to plant trait z_r is (van Tienderen 2000):

$$s_{z_r} = \frac{\partial \lambda}{\partial z_r} = \sum_k \left(\left(\sum_{ij} \frac{\partial \lambda}{\partial a_{ij}} \frac{\partial a_{ij}}{\partial w_k} \right) \frac{\partial w_k}{\partial z_r} \right) = \sum_k s_{w_k} \frac{\partial w_k}{\partial z_r} \quad (11)$$

$$e_{z_r} = \frac{z_r}{\lambda} \frac{\partial \lambda}{\partial z_r} = \frac{z_r}{\lambda} \sum_k s_{w_k} \frac{\partial w_k}{\partial z_r} \quad (12)$$

Whereas the elasticities of λ to all matrix elements sum to one (Mesterton-Gibbons 1993), this is not true for the elasticities of λ to the life history components or plant traits and elasticities of lower-level parameters can not be interpreted as relative contributions to λ (Caswell 2001). In order to be able to compare plant traits which differ strongly in their mean value we also calculated phenotypic selection gradients, β , which measure the proportional increase in λ per unit of z_r (van Tienderen 2000):

$$\beta_{z_r} = \frac{1}{\lambda} \frac{\partial \lambda}{\partial z_r} = \frac{z_r}{z_r} \frac{1}{\lambda} \frac{\partial \lambda}{\partial z_r} = \frac{e_{z_r}}{z_r} \quad (13)$$

Analyses of the impact of nutrient enrichment on the population dynamics

Besides a comparison of the elasticity values of the population dynamics to which the effects of enrichment were applied with that of undisturbed population dynamics, two other types of analyses were carried out. Firstly, we varied the six nutrient factors, f_i , one by one, while keeping the others constant. The effect on λ was investigated both in the range where the affected component or trait was lowered (f_i between 0 and 1) and in a range where the affected parameter is raised (f_i between 1 and 2.5).

Secondly we performed life-table response experiment analyses (LTRE) to decompose the differences in λ of the natural and enriched matrices into contributions by the differences in the matrix elements or lower-level parameters. The matrix of the natural population dynamics of each species, A^N , served as the reference matrix. We estimated the LTRE effect of nutrient enrichment in each species with the following formulas (Horvitz et al. 1997; Caswell 2001; Cooch et al. 2001):

Table 2.

The experimentally determined values of the relative impact of fertilization, f_i , on different plant traits and life history components. Besides the f -values of the four species (*Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*), the f -values of the natural population dynamics of all species are also given.

f_i	Affected trait/component		Field values	Fertilized values			
			All species	<i>H. radicata</i>	<i>S. pratensis</i>	<i>C. dissectum</i>	<i>C. jacea</i>
f_1	Plant size	Z_1	1.00	1.68	1.34	0.81	1.44
f_2	Flowering threshold	Z_2	0.00	0.00	0.00	0.00	0.48
f_3	Relative seed production	Z_3	1.00	1.93	2.48	1.00	2.02
f_4	Seedling survival	W_1	1.00	0.15	1.00	0.53	1.00
f_5	Adult survival	$W_{2,3,4,5}$	1.00	0.15	1.00	0.53	1.00
f_6	Establishment rate	W_{10}	1.00	1.00	0.31	0.00	0.00

$$\tilde{\phi} = \lambda^F - \lambda^N \quad (14)$$

$$\cong \sum_{ij} (a_{ij}^F - a_{ij}^N) \frac{\partial \lambda}{\partial a_{ij}} \bigg|_{\frac{1}{2}(A^F + A^N)} \quad (15)$$

$$\cong \sum_k (w_k^F - w_k^N) \frac{\partial \lambda}{\partial w_k} \bigg|_{\frac{1}{2}(A^F + A^N)} \quad (16)$$

$$\cong \sum_r (z_r^F - z_r^N) \frac{\partial \lambda}{\partial z_r} \bigg|_{\frac{1}{2}(A^F + A^N)} \quad (17)$$

in which the effect of nutrient enrichment, ϕ , is estimated (eq. 15) by summing over all matrix elements the product of the difference between the matrix elements of the fertilized and the control matrix, and the sensitivity of λ to that element, determined half-way the fertilized and natural matrix. In a similar way the difference in λ was decomposed into the difference in life history components (eq. 16) or the difference in plant traits (eq. 17).

Results

Different effects of nutrient enrichment in species differing in longevity and mode of reproduction

Nutrient enrichment had different effects on the four species studied. Sexual reproductive allocation and vegetative plant size of surviving plants both increased in three species, but not in *Cirsium dissectum* (Table 2). As predicted, nutrient

enrichment had a larger impact on the λ of the most short-lived species. This was mostly caused by the observed effects on survival in the nutrient enrichment experiment (Chapter 3; Table 2), as λ was very responsive to changes in survival rates in all four herbaceous perennials (Fig. 2e). The positive effects of fertilization on λ through increased plant sexual reproductive allocation and especially increased plant size did have the potential to compensate for moderate increases in mortality (Fig. 2a,c).

The importance of life history components and plant traits for population growth rate

Survival of non-flowering rosettes, w_2 , was the most important life history component in all species (Fig. 3e/h). This component became less important in the fertilized population matrix of *S. pratensis* and especially *H. radicata*, whereas only small changes took place in the elasticity values of life history components in the two clonal species. Life history components of sexual reproduction, w_1 , w_6 , w_9 and w_{10} , gained in importance in the declining, fertilized population of *H. radicata* (Fig. 3a,e). In *S. pratensis* however, the emphasis of the elasticity of λ shifted towards the relative importance of survival of flowering rosettes, w_3 , and the probability that those rosettes flower again one year later, w_7 .

Only the results of *H. radicata* were consistent with our expectation that plant size would be more important in fertilized population than in natural populations (Fig. 3i), while the selection gradients of plant size decreased in magnitude due to fertilization in the other species (Fig. 3j,k,l). Especially the part of the selection gradient of plant size that involved the flowering probability of vegetative rosettes, w_8 , was increased in the fertilized population of *H. radicata*. Although the trait elasticity values of plant size were much higher than that of sexual reproductive allocation, their selection gradients were of the same magnitude in *H. radicata* and *S. pratensis* (Fig. 3i,j). This is because the selection gradients only equal the elasticity values after division by the mean trait value, which is much larger in plant size than in sexual reproductive allocation. In the third species in which fertilization increased both size and allocation plant traits, *C. jacea*, the selection gradient of sexual reproductive allocation was much higher than that of plant size in the natural population, but was reduced to zero in the fertilized population where no recruitment occurred at all.

Changes in population dynamics due to nutrient enrichment

The population growth rate, λ , was strongly reduced by the effects of nutrient enrichment in *Hypochaeris radicata* (-66%; from $\lambda = 0.96$ to 0.33) and *Cirsium dissectum* (-50%; $\lambda = 1.01$ to 0.51) but only slightly affected in *Centaurea jacea* (-6%; $\lambda = 0.98$ to 0.92) and *Succisa pratensis* (+4%; $\lambda = 1.23$ to 1.28). The decomposition model fitted well as the summed LTRE-effect, ϕ , of the matrix elements resembled the difference in λ closely (the difference was -1.2% on average). The LTRE-effects of the

life history components deviated more from $\Delta\lambda$ (-3.5% on average), which is probably due to deviations that arise when sum of products of partial derivatives of lower-level parameters are compared with partial derivatives of matrix elements (see Appendix A in Cooch et al. 2001 for a discussion on this problem). The LTRE-effects of the plant traits did not resemble $\Delta\lambda$ at all (+4,900% on average). This has several reasons, of which the above-mentioned deviations, are one. But it is also because the differences between control and fertilized matrices are not only due to differences in plant traits but also due to differences in life history components directly. And lastly, the LTRE-effects of plant traits are strongly dependent on the scale of the traits involved.

The large reductions of λ in the two species with short-lived rosettes were mostly due to a lower adult survival rate but also due to decreased seedling survival (*H. radicata*; Fig. 4a,e) and less clonal propagation (*C. dissectum*; Fig. 4c,g). The contribution of sexual reproduction to $\Delta\lambda$ increased only in *H. radicata* (Fig. 4a) and was reduced most strongly in *C. jacea* (Fig. 4d).

One of our research questions was to investigate whether the populations of these herbaceous perennials become 'senile' or more dynamic when the grasslands they inhabit become more productive due to succession or nitrogen deposition. The stable stage structure of the populations changed in three of the four species: the proportion of seedlings in the strongly declining, fertilized *H. radicata* population was much higher than in the natural population (71% vs. 27%), probably due to the strongly increased rosette mortality rates (Table 2). Contrastingly, the proportion of seedlings was reduced from 6% to 0% in *C. jacea* while the proportion of new clonal offspring increased to 45%. The relative number of seedlings was more constant in *S. pratensis*, but in that species a higher percentage of the plants flowered under nutrient enriched conditions (23% vs. 16%). Overall, only the population of *C. jacea* seems to enter a senile phase without seedling recruitment, because *C. dissectum* had insignificant seedling recruitment already under unfertilized conditions and in the other two species lower recruitment rates are almost entirely compensated for by both higher flowering probabilities (positive LTRE-effect of w_6 ; Fig. 4e,f) and higher seed production per flowering rosette (positive LTRE-effect of w_9 , Fig. 4e,f).

The trait mean-standardized LTRE-effects show that the difference in sexual reproductive allocation contributed more to the difference in λ in *H. radicata* and in *C. jacea* (Fig. 4i,l), while the difference in plant size contributed relatively more in the other two species (Fig. 4j,k).

Discussion

The balance of positive, direct effects on plant traits and negative, indirect effects on some life history components by nutrient enrichment differed for short-lived and long-lived herbaceous perennials: our modeling exercise revealed that in short-lived species the negative effects of increased competition by neighbors prevailed by

strongly increasing mortality rate and consequently a lower population growth rate. In the species with longer-lived rosettes the mortality rates were unaffected, and the negative effects of reduced establishment rates were compensated for by the positive effects on seed production through increased plant size and increased sexual reproductive allocation. This suggests that when grassland herbs are being gradually suppressed during succession they can first maintain their population growth rate by compensating for lower seedling establishment chances. But when eventually mortality rates go up by continued increases in vegetation biomass also the populations of these longer-lived herb species will strongly decline.

In our previous experiment (Chapter 3) mortality rates were unchanged in the long-lived *Succisa pratensis* and *Centaurea jacea* after two years of nutrient enrichment, but the increased variation in plant size in *S. pratensis* at the end of the experiment may indicate that some plants were going to die in the fertilized plots if the experiment had lasted longer. Billeter et al. (2003) reported lower adult survival of *S. pratensis* after longer periods of nutrient enrichment in the field. Is this pattern of first a reduction in recruitment followed by a decrease of adult survival different from other studies? Oostermeijer et al. (1996) clearly show that in the long-lived herb *Gentiana pneumonanthe* populations in closed vegetations have a λ lower than one and almost no recruitment. The elasticity of fecundity was positively correlated with λ while the elasticity of survival increased with lower λ . But the fecundity elements were also positively correlated with important survival elements, indicating that both recruitment and survival was reduced in these declining populations. These authors however also suggested that it is recruitment that decreases first during succession. Besides the results on *S. pratensis* and *C. jacea*, the data on the rare *C. dissectum* may also be consistent with this general pattern, when considering that seedling recruitment was already prohibited in earlier phases of succession and that genets of this rhizomatous species are long-lived. The short-lived *H. radicata* however showed the opposite pattern: nutrient enrichment induced relatively more mortality than a reduction in recruitment, and the proportion of seedlings was found to increase rather than decrease in declining populations.

The importance of sexual reproductive allocation for the population growth rate

In the two mainly sexually reproducing species, *H. radicata* and *S. pratensis*, all elasticity values of the life history components that are part of the sexual reproduction pathway (flowering probability of vegetative rosettes, seed production, seedling recruitment and seedling survival) increased to some extent when fertilization was simulated. Still the importance of the allocation traits was limited, although the increased sexual reproductive allocation markedly increased seed production, which can be a significant step in the sexual pathway (Chapter 4). This may be caused by the limited contribution to λ by fecundity. Plant size in comparison mattered much more for λ as it is also part of the survival and clonal propagation components. But when analyzing only sexual reproduction, plant size has higher elasticity values than

sexual reproductive allocation because it not only influences seed production directly, but also determines whether rosettes will flower or not. Although the increased seed production (caused by increases both in size and allocation due to fertilization) may have limited buffering capacities for the overall negative effects of fertilization, it can be very important when locally small-scale disturbances are introduced and for regional population dynamics as the number of produced seeds is a very important aspect of the colonization capacity of these species (Soons et al. 2004).

Flowering threshold was the least important trait in our analyses. All but one relationship of sexual reproductive biomass and vegetative biomass in the garden experiment (Chapter 3) went through the origin, indicating that a flowering threshold size plays a minor role and is hard to determine (Klinkhamer et al. 1992). The logistic regressions of plant size on flowering probabilities in the field showed no clear-cut flowering thresholds either, although it may be that rosette size is not the best estimator (but one of the most feasible of the non-destructive options) of the biomass of a plant like *S. pratensis* (Graaskamp 2003).

Transition matrices as tools for studying the population consequences of effects on vital rates

The benefits of a model in which demographic field data and results of manipulative experiments are combined are that the importance of the experimental findings for populations can be evaluated and that long-term effects can be modeled (Gotelli & Ellison 2002). Elasticity and LTRE provided two powerful means to analyze population matrices. Both methods answer different questions (Caswell 2000), but can supplement each other in a useful way: elasticity values indicate on which parameters λ depends, while LTRE analyses which deviations in different parameters caused the observed variation in λ . When the natural variation in population dynamics is studied (Chapter 5) elasticity values and LTRE are positively correlated because they are based on the same components: the sensitivity value and the mean (or the positively correlated variation) of the matrix elements.

Hierarchically constructed models further allow for similar analyses of the underlying life history components (van Tienderen 2000; Cooch et al. 2001). This opens the opportunity to investigate trade-offs between model components, and their effect on the population dynamics (van Tienderen 2000; Shefferson et al. 2003). However, negative correlations in elasticity patterns can not be directly interpreted as trade-offs due to their relative nature (Shea et al. 1994; de Kroon et al. 2000). Ehrlén (2002) further warns that trade-offs may involve several consecutive years. The best way to study trade-offs is probably to use knowledge of the correlations of the underlying plant traits, and to evaluate direct and indirect elasticity values of these traits (van Tienderen 2000). However, we assumed that the plant traits in our model were independent and that the important trade-offs between life history components in our species (Chapter 2) were inherent to the demographic field data.

Conclusions

Nutrient enrichment has strong negative influences on the population growth rate of herbaceous perennials in grasslands. In long-lived species it may take a few years before adult survival is affected but also populations of these species will face serious extinction risks when succession progresses or nutrients continue to enter the system. The investigated species have some capacity to buffer lower establishment and survival by increased seed production through higher sexual reproductive allocation and larger plant sizes, but when survival rates drop further the population growth rates will decline quickly. In the end increased seed production is only a feasible life history strategy if it leads to a higher life time reproductive success.

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Summary

This study aims to contribute to the knowledge of how plants respond to adverse influences of intensified land use. In particular, attention was paid to the ways in which life history strategies change in order to buffer environmental variation, and which important parts of the life cycle are affected most. Herbaceous plant species are often threatened when the land use in an agricultural landscape is intensified: habitat is destroyed for new fields, infrastructure or urban areas, or habitat becomes less favorable because water levels are lowered for agriculture and because of pollution and nutrient enrichment by the influx of particles through air and water from the neighboring fields. Nutrient enrichment enhances natural succession and causes more competitive species to take over from species that are characteristic for nutrient-poor grasslands. As a result of habitat reduction and deterioration, populations decline in size and are more prone to extinction. The distances between remaining populations also increases, which makes gene flow through pollen or seed dispersal less likely. Decreased gene flow may lead to inbreeding depression and again higher extinction risks.

Plant species may counterbalance some of these influences by flexible growth patterns and may adapt for instance to nutrient enrichment. For the conservation of the floral diversity of agricultural landscapes it is therefore important to know to what extent plant species are endangered by the different effects of intensified land use and to what extent these species can buffer the changes in their growing conditions. In this study I concentrate on four co-occurring perennial herbs in nutrient-poor, species-rich meadows. With three species from the Asteraceae (*Cirsium dissectum* (L.) Hill, *Hypochaeris radicata* L. and *Centaurea jacea* L.) and one from the Dipsacaceae (*Succisa pratensis* Moench), the species are phylogenetically related but have contrasting life history strategies as they differ in the life-span of their rosettes and in the rate of clonal propagation.

The flexibility of the allocation of biomass to different life history functions (plant growth, sexual reproduction and clonal reproduction) is investigated in chapter 2. In a one-season garden experiment we continuously removed either rosette buds or flower buds to study the responses to damage. All investigated plants compensated for the removed buds. The short-lived species (*H. radicata*) showed stronger compensation for lost flower buds than the longer-lived species (*S. pratensis* and *C. jacea*) and the species with a high clonal propagation rate (*C. jacea*) compensated more strongly for lost rosette buds than the infrequently ramifying species. However, two species (*H. radicata* and *S. pratensis*) also switched to increased rosette formation when flower

buds were removed. This switch shows, together with increased vegetative plant weight, that sexual reproduction has costs in terms of growth in size and clonal propagation.

Costs of sexual reproduction underlie the hypotheses on the response of plants that are gradually outcompeted during succession: plants are expected to either increase their seed production to escape to other sites through seed dispersal (but at the expense of plant growth and future reproduction), or alternatively to persist by investing more in vegetative growth. In chapter 3 we aim to test these long-standing, but largely untested hypotheses in a three-year garden experiment. The target plants were grown together with a dominating, tall grass (*Molinia caerulea* (L.) Moench). Costs of sexual reproduction were investigated by continuous flower bud removal and were found in all target species as either reduced plant biomass or reduced number of rosettes. Succession was mimicked by fertilizing of the plots, which caused a threefold increase of the grass biomass. Mortality rates increased under fertilization in two target species (*H. radicata* and *C. dissectum*) probably because they lacked a means of competing with the grasses. Three target species increased their relative allocation to flowers and seeds when their plots were enriched, while their relative allocation to storage did not decrease but in some species even slightly increased. In the fourth species (*C. jacea*) seed production was only higher in large plants but not in small plants. Allocation to every life history function was strongly and positively dependent on plant size. Consequently, the two hypothesized strategies collapse into a single syndrome: in response to succession plants must increase in size in order to survive and to increase seed production.

The sexual reproduction pathway, from flower production to established seedlings, is further investigated in chapter 4. By sampling flowering plants and flower heads with seeds and by performing seed addition experiments we quantified for each species all involved steps: flower head production, flower production, seed set, seed predation and establishment as seedlings. The two infrequently ramifying species (*H. radicata* and *S. pratensis*) had high numbers of seedlings per flowering rosette: more than one on average. This number was below one in the two more clonally propagating species (*C. dissectum* and *C. jacea*). Seedling establishment was the largest bottleneck in the pathway, while seed set was important too in one of the more clonal species. The different steps were studied in several sites and over several years. The spatiotemporal coefficients of variation were highest for seedling establishment, but closely followed in magnitude by the coefficients for flower head production and seed set, indicating that selection processes can be expected in different parts of the sexual pathway of the life cycle.

The whole life cycle is studied for three species (*S. pratensis*, *H. radicata* and *C. jacea*) in chapter 5. Demographic measurements on rosette survival, clonal propagation and flower head production were made in permanent plots in three or five populations over four years. The sexual pathway of the life cycle was completed with data of the

previous chapter. We constructed 42 stage-based projection matrices to study the population dynamics of the three species in different sites over time. The calculated population growth rates were lowest in the most short-lived species (*H. radicata*) and most stable in the most clonal species (*C. jacea*). Using life table response experiment analyses we found that the species differed in their response to the same spatiotemporal variation in environmental conditions. Furthermore, species responded differently in sites in which they had low population growth rates than to years in which they had low population growth rates. In two species (*S. pratensis* and *H. radicata*) below-average values of some life cycle components in bad sites were partly compensated for by the above-average values of other components, but similar compensation did not occur in bad years. By contrast, this compensation did appear in bad years for the third, more clonal species (*C. jacea*). The implication of this difference between spatial and temporal variation in population dynamics is that temporal variation can not readily be substituted with spatial variation as has previously been done in some time-limited studies.

In chapter 6 we investigate the demographic implications of inbreeding depression in order to evaluate if reported effects of inbreeding on several life history components significantly influence the population dynamics and population survival of *S. pratensis*. For that purpose we constructed a projection matrix model with an outcrossing and an inbreeding cycle. The growth of seedlings and small rosettes were reduced in the latter cycle, as was seed set and germination, using empirically based data. The proportion of seeds entering either cycle was a function of the size of the population: more seeds were inbred in smaller populations. We compared a declining and an increasing population in the simulations. Although only a part of the life cycle was affected, inbreeding reduced the time to extinction in the decreasing population. Inbreeding affected the increasing population only when the initial population was small. Density dependency by limiting habitat size only affected the increasing population.

The demography of the most clonal (rhizomatous) and also most endangered herb of the model species (*C. dissectum*) was studied in chapter 7. The results of the allocation experiment of the third chapter are further analyzed: whereas the total biomass of the plants was unaffected by the fertilization treatment, the surviving rosettes were further away from the original planting location and more remnants of dead rosettes were found in the fertilized plots. Field observations on excavated plants and an additional experiment confirmed that both flowering and rhizome formation are size-dependent. This suggests that the high percentage of flowering rosettes in the allocation experiment was the result of a high growth rate of the fertilized rosettes. The total biomass did not differ from the control plots because the mortality rate of the non-flowering rosettes was also higher in the fertilized plots. This pattern of increased flowering and of increased turn-over of biomass and rosettes is consistent with the results of a demographic field study. In the most productive site we found high flowering probabilities and above-average clonal propagation but lower rosette survival. This trend towards higher turn-over rates makes this bad competitor even

more prone to extinction when the nutrient enrichment of a meadow continues. The increased seed production has a negligible contribution to the local population growth rate as seedling recruitment seems confined to very open, early-successional habitats.

The last chapter was aimed to integrate the population dynamics models and the results of the fertilization experiments to investigate which effects on individual plants have the strongest impact on the dynamics of the population. As fertilization had both positive effects on plant size and seed production and negative effects on survival and seedling establishment, we were also interested in the overall outcome when all effects were incorporated into a model describing the natural population dynamics. In order to model effects on life history components and plant traits we built a hierarchical matrix in which each element was a function of life history components and many of the components in turn were functions of plant traits. The negative effects of fertilization on the population growth rate by increased mortality rates in the two weak competitors (*C. dissectum* and *H. radicata*) overruled other effects and caused strong population declines. In the other two species (*S. pratensis* and *C. jacea*), in which mortality was still unaffected, negative impacts of reduced seedling establishment were small or compensated by increased seed production and plant size. However, the model results also suggest that also in these better competitors populations will decline once the mortality rates of individuals increase. Positive effects on plant size and seed number can not compensate for high death rates. We therefore conclude that although the life history responses of these perennials are rather flexible, their populations will go extinct in grasslands that have become more productive through succession and nutrient enrichment. The decline of small population can be expected to be even accelerated by inbreeding depressions. However, when increased allocation to sexual reproduction leads to increased seed production for the total population, this flexible life history response may have positive effects on the dynamics of a species in the landscape through increased colonization probabilities.

Samenvatting

Het doel van deze studie is bij te dragen aan de kennis over de wijze waarop wilde planten reageren op de negatieve effecten van een intensiever landschapsgebruik door mensen. Onderzocht is vooral de manier waarop planten hun levensstrategieën veranderen om de verandering in de groeiomstandigheden te bufferen, en welke belangrijke onderdelen van de levenscyclus het meest beïnvloed worden door het veranderde milieu. Kruiden worden vaak bedreigd wanneer de landbouw in een landschap wordt geïntensiveerd: habitat wordt vernietigd voor nieuwe akkers, infrastructuur of bebouwing, of de kwaliteit van de habitat gaat achteruit doordat de grondwaterstand wordt verlaagd voor de landbouw en doordat graslanden vervuild en verrijkt worden door de omgevende landbouw via het water en de lucht. Verrijking versterkt natuurlijke successie van de vegetatie en zorgt ervoor dat meer competitieve soorten gaan domineren ten koste van soorten die karakteristiek zijn voor nutriëntarme graslanden. Door habitatverkleining en -verslechtering worden plantenpopulaties kleiner, waardoor de kans op uitsterven groter wordt. De afstand tussen populaties wordt groter bij intensiever landgebruik, waardoor pollen en zaden minder makkelijk van de ene naar een andere populatie worden verspreid. Daardoor ontstaat inteelt, wat de vitaliteit van de planten kan benadelen en de kans op uitsterven van een populatie wederom vergroot.

Planten zouden een aantal van deze invloeden kunnen compenseren met hun flexibele groei en zich kunnen aanpassen aan voedselrijke omstandigheden. Voor het behoud van de floristische diversiteit in agrarische landschappen is het daarom belangrijk te weten hoezeer plantensoorten bedreigd worden door intensief landgebruik en in welke mate deze soorten de veranderingen in het milieu kunnen opvangen met aanpassingen. In deze studie staan vier kruiden centraal, die samen voorkomen in nutriëntarme maar soortenrijke hooilanden. De soorten zijn fylogenetisch verwant (drie Asteraceae: Spaanse ruiter (*Cirsium dissectum* (L.) Hill), Gewoon biggekruid (*Hypochaeris radicata* L.) en Knoopkruid (*Centaurea jacea* L.) en een Dipsacaceae: Blauwe knoop (*Succisa pratensis* Moench)), maar ze hebben contrasterende levensstrategieën: de levensduur van de rozetten en de frequentie van uitstoeling verschillen tussen de soorten.

De flexibiliteit van de biomassa-allocatie naar verschillende plantonderdelen, die van belang zijn voor de contrasterende levensstrategieën (groei van het rozet, seksuele reproductie en klonale vermeerdering), wordt beschreven in hoofdstuk 2. We verwijderden de knoppen van ofwel bloemhoofdjes ofwel zijrozetten gedurende een groeiseizoen in een experiment in een proeftuin om uit te vinden hoe de planten

reageren op dergelijke beschadiging. Alle planten compenseerden voor de continu verwijderde knoppen. Maar het kortlevende *H. radicata* compenseerde meer voor verwijderde knoppen van bloemhoofdjes dan de langer levende *S. pratensis* en *C. jacea*, en de soort met frequente klonale vermeerdering, *C. jacea*, compenseerde meer voor verwijderde zijrozetjes dan de soorten met infrequentere uitstoeling. *Hypochaeris radicata* en *S. pratensis* maakten echter ook meer nieuwe rozetten wanneer de bloemknoppen continu verwijderd werden. Deze verschuiving laat, samen met de grotere plantgewichten ten opzichte van de niet behandelde controleplanten, zien dat seksuele reproductie duidelijk ten koste gaat van de groei van het rozet en de klonale vermeerdering.

Deze kosten van seksuele reproductie vormen het uitgangspunt van hypothesen over de respons van planten die in de verdrukking komen tijdens successie: planten worden verondersteld ofwel hun zaadproductie te verhogen (hetgeen dus wel ten koste gaat van groei en toekomstige zaadproductie) om zo te ontsnappen via zaadverspreiding, ofwel de verhoogde competitie met andere planten te weerstaan door juist meer te investeren in vegetatieve groei. In hoofdstuk 3 toetsten we deze oude, maar nog weinig geteste hypothesen in een driejarig experiment in de proeftuin. Individuele stekjes van de vier soorten werden geplant tussen pollen van het hoogopgaande en dominerende gras Pijpenstrootje (*Molinia caerulea* (L.) Moench). De kosten van seksuele reproductie werden onderzocht door continue knopverwijdering en werden bij alle soorten aangetroffen als afgenomen plantgewicht of lager rozetaantal. Successie werd nagebootst door de plotjes te verrijken, waardoor het gras driemaal zo zwaar werd. Tegelijkertijd nam de sterfte toe bij de *C. dissectum* en *H. radicata*, waarschijnlijk doordat deze soorten niet kunnen concurreren met het gras. Drie doelsoorten verhoogden hun relatieve biomassa-allocatie naar de bloemen en zaden wanneer de plots verrijkt werden, terwijl hun relatieve allocatie naar de opslagorganen niet verminderde maar in sommige soorten zelfs lichtelijk toenam. Bij de vierde soort, *C. jacea*, was de zaadproductie alleen hoger bij grote planten, maar niet bij kleinere. Maar bovenal was allocatie naar de verschillende onderdelen van de planten afhankelijk van de grootte van de plant: alle onderdelen van grote planten zijn zwaarder dan die van kleine planten. Daarom vallen de twee contrasterende hypothesen samen: in latere successiestadia van de vegetatie moeten planten groter worden om nog te kunnen overleven maar ook om meer zaden te kunnen produceren.

De seksuele reproductie bij planten, van bloei tot zaailing, wordt verder onderzocht in hoofdstuk 4. Alle fasen in dit proces (het aanmaken van bloemhoofdjes, bloemen en zaden, zaadpredatie en de vestiging van zaailingen) werden gekwantificeerd door bloeiende planten te bemonsteren, door bloemen en zaden te tellen in afzonderlijke bloemhoofdjes en door zaai-experimenten. De twee soorten die relatief weinig uitstoelen, *H. radicata* en *S. pratensis*, hadden gemiddeld meer dan 1 zaailing per jaar per bloeiend rozet. Dit getal kwam echter veel lager uit dan 1 bij de meer klonale soorten *C. jacea* en vooral *C. dissectum*. De grootste verliezen traden op in de vestigingsfase in alle soorten, maar ook bij de zaadontwikkeling in *C. dissectum*. De

verschillende fasen van de seksuele reproductie waren in verschillende jaren en gebieden onderzocht. De variatiecoëfficiënten waren het hoogst in de vestigingsfase, maar ook in de fase van de bloemhoofdproductie en bij het ontwikkelen van het zaad. Dit suggereert dat selectie kan plaatsvinden in verschillende fasen van de seksuele reproductie.

De gehele levenscyclus van *S. pratensis*, *H. radicata* en *C. jacea* wordt beschreven bestudeerd in hoofdstuk 5. De overleving van rozetten, vegetatieve vermeerdering en de productie van bloemhoofdjes werden bepaald in permanente kwadraten in drie tot vijf populaties in vier achtereenvolgende jaren. De seksuele-reproductiefase van de levenscyclus werd compleet met data uit het voorgaande hoofdstuk. In totaal stelden we 42 projectiematrices met phenologische klassen samen om de populatiedynamiek van de drie soorten te onderzoeken in de verschillende gebieden en jaren. De berekende populatie-groeisnelheden waren het laagst bij de kortstlevende soort (*H. radicata*) en het meest stabiel in de meest klonale soort (*C. jacea*). Variantie decompositie technieken voor matrices toonden dat de soorten verschillend reageerden op dezelfde ruimtelijke en temporele variatie in groeiomstandigheden. Bovendien reageerden de soorten verschillend in gebieden waarin ze lager dan gemiddelde populatie-groeisnelheden hadden dan in jaren waarin ze lagere populatie-groeisnelheden hadden. Bij *S. pratensis* en *H. radicata* werden de lage waarden van sommige componenten van de levenscyclus in slechte gebieden gedeeltelijk gecompenseerd met hoge waarden van andere componenten, maar dergelijke compensatie werd niet gevonden in slechte jaren. Daarentegen werd compensatie in slechte jaren wel gevonden in *C. jacea*. Dit verschil tussen ruimtelijke en temporele variatie in de populatiedynamiek impliceert dat temporele variatie niet zomaar vervangen kan worden met ruimtelijke variatie zoals soms gebeurt in tijd-gelimiteerde studies.

In hoofdstuk 6 onderzoeken we de demografische implicaties van de negatieve effecten van inteelt, om te evalueren of gerapporteerde effecten van inteelt op bepaalde onderdelen van de levenscyclus een significante invloed hebben op de populatiedynamiek en -overleving van *S. pratensis*. Daarvoor construeerden we een projectie matrix model met een kruisbestuivingscyclus en zelfbestuivingscyclus. De zaadproductie, kieming en groei van zaailingen en kleine rozetten werd verkleind in de inteeltcyclus op basis van inteeltexperimenten. Het percentage zaden dat in de inteeltcyclus kwam was een functie van de populatiegrootte: meer zaden waren ingeteeld in kleinere populaties. In de simulaties vergeleken we een afnemende populatie met een populatie die toenam in aantal planten. Hoewel slechts een deel van de levenscyclus werd beïnvloed, reduceerde inteelt de tijd tot uitsterven in de afnemende populatie. De toenemende populatie werd enkel beïnvloed door inteelt bij kleine initiële populatiegrootte in de simulaties. Dichtheidsafhankelijkheid door limiterende habitat-grootte had enkel effect op de toenemende populatie.

De demografie van de meest klonale en ook meest bedreigde van de bestudeerde soorten (*C. dissectum*) wordt bestudeerd in hoofdstuk 7. De resultaten van het allocatie-experiment van het derde hoofdstuk werden verder geanalyseerd voor deze soort: hoewel de totale biomassa van de planten niet beïnvloed werd door de verrijkingsbehandeling, waren de overlevende rozetten verder weg van de plek waar de eerste plant stond en waren er meer resten van dode rozetten gevonden in de verrijkte plots. Veldwaarnemingen aan uitgegraven planten en een extra kasexperiment bevestigden dat zowel bloei als de vorming van wortelstokken afhankelijk zijn van de grootte van een rozet. Dit suggereert dat het hoge percentage van bloeiende rozetten in het allocatie-experiment het resultaat was van een grotere groeisnelheid in de verrijkte plots ten opzichte van de niet verrijkte plots. De totale biomassa van *C. dissectum* verschilde niet tussen de twee behandelingen omdat vegetatieve rozetten ook sneller stierven in de verrijkte plots. Dit patroon van meer bloei en verhoogde biomassa-roulatie is consistent met de resultaten van een demografische studie in natuurgebieden. In het meest productieve gebied vonden we hoge bloeikansen en bovengemiddelde vegetatieve vermeerdering maar lagere overleving van rozetten. Deze trend naar hogere roulatie van rozetten maakt deze weinig-competitieve soort nog gevoeliger voor uitsterven wanneer de verrijking van zijn habitat toe blijft nemen. De verhoogde zaadproductie heeft een verwaarloosbaar effect op de lokale populatie groeisnelheid omdat het vestigen van zaailingen beperkt lijkt te zijn tot graslanden met zeer veel open plekken.

In het laatste hoofdstuk wordt geprobeerd de populatiemodellen en de resultaten van het bemestingsexperiment te integreren om te onderzoeken welke effecten van bemesting op individuele planten de grootste invloed hebben op de populatiedynamiek. Omdat bemesting zowel een positieve invloed heeft op plantgrootte en zaadproductie als een negatieve invloed op de overleving en de vestiging van zaailingen, waren we ook geïnteresseerd in het totale effect van bemesting wanneer alle afzonderlijke effecten meegenomen worden in de populatiemodellen die de natuurlijke dynamiek beschrijven. Om de effecten van afzonderlijke levenscycluscomponenten en planteneigenschappen te modelleren, bouwden we een hiërarchisch matrixmodel, waarin elk element een functie was van een of meer componenten van de levenscyclus en waarin veel van die componenten functies waren van planteneigenschappen. Bij de twee weinig competitieve soorten (*C. dissectum* and *H. radicata*) overheersten de negatieve effecten van bemesten op de populatie-groeisnelheid door verhoogde mortaliteit en werden de populaties snel kleiner. Bij de twee andere soorten (*S. pratensis* and *C. jacea*), waarin de mortaliteit nog niet beïnvloed werd, was de negatieve impact van een lagere vestigingskans van zaailingen klein of gecompenseerd door de toegenomen zaadproductie en plantgrootte. De modelresultaten suggereren echter wel dat ook van deze meer competitieve soorten de populatiegroeisnelheid snel zal dalen zodra de sterftesnelheid van individuen omhoog gaat. De positieve effecten op plantgrootte en op het aantal geproduceerde zaden kunnen de negatieve effecten van verhoogde mortaliteit niet opvangen. Daarom concluderen we dat, hoewel de levenscyclus van

deze perenne soorten redelijk flexibel is, hun populaties ten dode opgeschreven zijn in graslanden waarin de biomassaproductie van de vegetatie toeneemt door successie en verrijking. Bovendien zal de afname in aantallen planten waarschijnlijk versneld worden door de negatieve effecten van inteelt. Echter, indien een verhoogde allocatie naar seksuele reproductie resulteert in een verhoogde zaadproductie van de gehele populatie, kunnen deze flexibele responsen van de levenscyclus positieve effecten hebben op de metapopulatiedynamiek op landschapsschaal door verhoogde kolonisatiekansen.

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Curriculum vitae

Eelke Jongejans was born in Bovenkarspel, the Netherlands, on November 21st 1975. After attending the Marmellius gymnasium in Alkmaar for his secondary education, he studied biology at Wageningen University from 1994 till 1999. His master's project was on seed dispersal by wind in grassland plants (supervised by Dr. Peter Schippers). As a follow-up of the lab experiments and model exercises he went to Stockholm University to study seed dispersal in the field (supervised by Dr. Anders Telenius).

His Ph.D. project on the establishment, dynamics and extinction of populations of perennial plants in agricultural landscapes started in 1999 and was part of the 'Survival of Plant Species in Fragmented Landscapes' program funded by the Netherlands Organization for Scientific Research (NWO). In this project he combined demographic field observations and experiments on biomass allocation and seedling establishment with projection matrix model exercises (supervised by Prof. Frank Berendse of the Nature Conservation and Plant Ecology group of Wageningen University and by Prof. Hans de Kroon of the Experimental Plant Ecology section of the Radboud University Nijmegen). He participated in several Ph.D. courses on life history theory, spatial modeling and population dynamics and conservation. He gave oral presentations of his research at large conferences in Basel, Prague and York and presented posters at several others. During his Ph.D. project he supervised eight students on their M.Sc. thesis and assisted in teaching several courses for undergraduate students.

In 2004 he started a two-year research project on the demography and dispersal of invasive *Carduus* thistles at the Pennsylvania State University (at the laboratory of Dr. Katriona Shea). His main scientific interests are in processes at the plant level, and in the influence of those processes on the population dynamics of these plants, both at the local and at the regional scale. A few examples of such processes are adaptation to local environmental conditions competition, herbivory, infection by mycorrhizal fungi, plant developmental programs, resource allocation and seed dispersal.

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