

# Relationship between abundance and fecundity in the endangered grassland annual *Euphrasia rostkoviana* ssp. *fennica*

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We examined 18 populations of the endangered *Euphrasia rostkoviana* ssp. *fennica* situated in managed semi-natural grasslands or gravelled road verges in ten extant localities in south-eastern Sweden. The major threat to the subspecies is the steady decrease in habitat area and quality. In each population, abundance and fecundity of individuals were examined. Local factors (soil pH, litter-layer depth and vascular species richness) and isolation were also measured. We specifically wanted to examine if there was a positive relationship between abundance (population size and density) and fecundity in the investigated taxon. Larger populations of *E. rostkoviana* ssp. *fennica* were found to be denser. Population size and density were both significantly positively related to fecundity. A higher variability in seed production was also detected for individuals in sparse populations. An Allee effect, i.e. a reduced fecundity in small and sparse populations, could thus be demonstrated for the already strongly threatened subspecies.

Key words: Allee-effect, habitat fragmentation, hemi-parasite, population size and density, road verge, semi-natural grassland

## Introduction

One of the most dramatic landscape changes during the 20th century in Sweden and in most of Europe alike, is the rapid reduction in semi-natural grassland area in favour of arable land, woodland and abandonment (Bernes 1994). The changed land-use has resulted in an extensive fragmentation of the remaining grassland habitats, imposing a great threat on numerous plant and animal species (Gärdenfors 2005). Semi-

natural grasslands, including both meadows and unfertilised pastures, are characterized by a high species richness, with up to 40–60 species per m<sup>2</sup> (Kull & Zobel 1991, Eriksson & Eriksson 1997). The traditional land-use, which consists of mowing and livestock grazing, inhibits the successional progression and thus prevents many grassland specialists from being competitively excluded. This continuous disturbance is generally considered to be one of the most important premises for the persistence of many short-lived

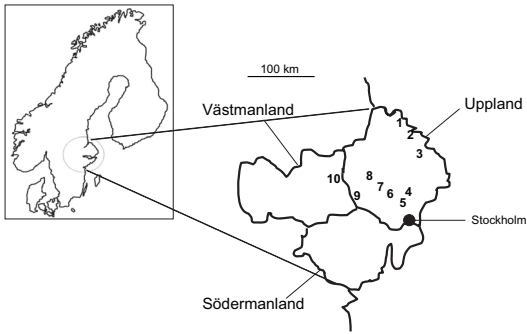
species in semi-natural grasslands, and the maintenance of a high local species diversity in these habitats (Eriksson 1996, Ekstam 2000).

The process of habitat fragmentation results primarily in two separate effects which both may have a major impact on the dynamics of plant populations. First, an increased distance between habitats makes dispersal of propagules more difficult. Second, habitat area will decrease and an area-related reduction in population size will lead to smaller and more vulnerable populations (Hanski 1999, Caswell 2000). The remaining habitat fragments may also experience an accelerated quality degradation, e.g. via increased edge effects, making the fragmented populations even more vulnerable to extinction (Saunders *et al.* 1991, Wiens 1997, Harrison & Bruna 1999, Kiviniemi & Eriksson 2002). Numerous plant species in semi-natural grasslands experience isolation to such a degree that new colonisation events hardly ever occur (Eriksson & Ehrlén 2001).

An isolated small population is exposed to elevated risks of local extinction as a consequence of increased genetic drift, inbreeding depression, sensitivity to demographic and environmental stochasticity and disrupted biotic interactions (e.g. Ellstrand & Ellman 1993, Lienert 2004). The combined negative effect of genetic drift and inbreeding leads to genetically eroded populations which suffer from a higher degree of homozygosity, i.e. a higher proportion of deleterious recessive alleles manifested at a certain locus (Ouborg *et al.* 1991, Dudash & Fenster 2000). Genetic erosion may result in a decreased reproductive output and hence a reduced population viability (e.g. Ouborg & van Treuren 1995, Young *et al.* 1996). Populations may respond differently to low levels of genetic diversity depending on earlier history. Populations that decrease gradually or that have been small for a relatively large number of generations may experience little inbreeding depression, since selection tends to purge those populations of deleterious alleles (Lienert 2004). Apart from the genetic consequences of a small population size, plant mutualistic interactions with seed dispersers and pollinators may be affected by fragmentation (Jennersten 1988, Ouborg & van Treuren 1995,

Mustajärvi *et al.* 2001). For instance, pollinator foraging behaviour is often, according to the optimal foraging theory, directed towards large and not too distantly located food sources (Sih & Baltus 1987). In small or less dense plant populations, where the nectar or pollen is not abundant enough, pollinator diversity may also be reduced (Rathcke & Jules 1993). The negative effects associated with reduced densities or population sizes are broadly referred to as the Allee effect (Courchamp *et al.* 1999, Stephens *et al.* 1999, Lienert 2004). The Allee effect (after W.C. Allee) has been defined as a positive relationship between any component of individual fitness and either numbers or density of conspecifics (Stephens *et al.* 1999). The Allee effect, where fitness is reduced when population size or density is low, and which may result in depressed or negative per-capita growth rates (e.g. Courchamp *et al.* 1999), is surely of interest for endangered, rare or dramatically declining species (Lienert 2004, Leimu *et al.* 2006).

In this study, all localities of the annual hemiparasitic grassland herb *Euphrasia rostkoviana ssp. fennica* known since 1978 were inventoried in one of the subspecies two core areas situated in the Provinces of Uppland and Västmanland in southeastern Sweden. The subspecies represent the most common form of the taxon *Euphrasia rostkoviana*, but is still seriously threatened by extinction. Continuously grazed low nutrient grasslands and frequently mown gravelled road verges are the two habitat types known for the taxon. The subspecies is absent from the seed bank and is therefore depending on yearly recruitment by seeds for its persistence (Karlsson 1982). A total of 18 populations of *Euphrasia rostkoviana ssp. fennica* were studied in ten extant localities. We specifically wanted to examine if there was an Allee effect present in the investigated taxon, i.e. if there was a positive relationship between fecundity and abundance (numbers and density of conspecifics) (cf. Stephens *et al.* 1999). Fecundity of individuals was examined as the total number of fruits produced per individual and the mean number of seeds produced per fruit. We also investigated local factors, describing habitat quality, and distance to nearest population (isolation).



**Fig. 1.** The 18 populations were studied in ten localities (1–10) situated in the Provinces of Uppland and Västmanland in southeastern Sweden.

## Materials and methods

### Study species

The summer annual hemiparasite *Euphrasia rostkoviana* ssp. *fennica* (Orobanchaceae), a subspecies of *E. rostkoviana*, recently has a highly limited distribution in eastern Sweden (Schmalholz 2006). It is classified as “strongly threatened” in the Swedish red list for rare and threatened species (Gärdenfors 2005), and its present distribution is restricted to around 30 localities (K. Antonsson & M. Edqvist pers. comm.). The subspecies’ historical distribution consists of around 60 recorded localities with the highest concentrations of localities divided into two core areas (Karlsson 1982). One core area is situated at the boundary zone between the Provinces of Småland and Östergötland with about 20–25 historical localities and the other core area is located in the Provinces of Uppland and Västmanland, where around 35 historical localities are known. Its global distribution includes southern Finland, the Baltic region and central Russia (Hultén 1971). The taxon *E. rostkoviana* is morphologically characterised by the long multicellular, glandular hairs on the calyx and on the lower side of the floral leaves. This character is absent in other Swedish *Euphrasia* species (Karlsson 1982). Further, the subspecies *fennica* differs from the subspecies *rostkoviana* — the other subspecies in Sweden — in the number of teeth on one side of the lower floral leaves, ranging from 5–8 in *fennica* as compared with 4–6 in *rostkoviana*. Hybridization between subspecies

has not been recorded, perhaps due to the differences in habitat preferences.

The presence of a potential host perennial root system in the vicinity of the seedling is, due to the root parasitic habit, a prerequisite for sufficient growth and floral development. Common grassland species such as *Trifolium repens*, *Festuca ovina* and *Anthoxanthum odoratum* can all function as suitable hosts for *E. rostkoviana* ssp. *fennica* (Karlsson 1982). The subspecies is self-compatible and protogynous, i.e. the anthers usually open one day after the stigmas are being receptive, and the female and male reproductive organs are also spatially separated. The flowering period extends from July to August and the subspecies is cross-pollinated by bumblebees (usually *Bombus* sp.) and various *Syrphidae* flies (Karlsson 1982). The ovary develops into a two-celled capsule containing between 5 and 30 seeds (pers. obs.). The seeds of the subspecies are fairly small and lack dispersal enhancing structures like wings or hooks (Karlsson 1982). Common habitats for *E. rostkoviana* ssp. *fennica* are continuously disturbed, low nutrient grasslands. Road verges, with similar abiotic conditions, may play an important role as both rescue sites for local populations and as possible dispersal corridors (e.g. Kiviniemi & Eriksson 1999, Murphy & Lovett-Doust 2004, Cousins 2006). All species belonging to the genus *Euphrasia* are especially vulnerable due to absence of seed banks and persistent vegetative life-cycle stages (Aronsson 1999).

### Study sites

All localities known since 1978 were inventoried in one of the core areas, 18 situated in the Provinces of Uppland and 1 in the County of Västmanland. The subspecies was found in 10 localities (Fig. 1), with altogether 26 populations (13 populations were found in road verges). Totally 18 populations were chosen to be included in this study, of which 16 were situated in Uppland and 2 in Västmanland. Twelve populations were situated in semi-natural grasslands and the remaining eight populations were located in road verges. All grassland habitats were moderately grazed and the road verges were regularly

(at least yearly) mown. The landscape in the study area is dominated in the north by a mainly coniferous forest with arable fields occurring as isolated spots. Arable fields and different types of fodder grasslands dominate the central and southern parts of Uppland. Although the landscape composition differs between the northernmost and central/south parts, all populations included in this study were situated in a fairly equal landscape (i.e. surrounded by arable land as well as coniferous forests).

## Field study

### Abundance and fecundity

The field study was conducted during July to September 2005. The number of individuals in each population was counted (population size). The density was measured by counting the numbers of individuals in three randomly placed 5-dm<sup>2</sup> plots. Population was defined as a group of individuals in an area standing at least 50 meters apart from the next group of individuals. GPS points were sampled in all populations and the positions were mapped using a geographical information system (GIS). In each population, 20 individuals were marked, except in the smallest population in which 15 individuals were marked. The number of flowers and branches and the height of the selected individuals were measured. In September (during a week), fecundity was recorded in the populations. The number of fruits, i.e. capsules, was counted for every marked individual in each population. Also, the number of seeds in five randomly chosen capsules per individual was recorded.

### Local factors and isolation

Soil pH, litter-layer depth and total number of vascular plant species were measured in each population. pH was measured in two soil samples taken at a depth of 5–6 cm within the populations and analysed using a standardized pH-method (Swedish Standard, SS-ISO 10390). The pH values obtained were in accordance with measurements found in other studies (c.f. Cous-

ins & Eriksson 2002). Litter-layer depth was assessed in three samples and was determined by measuring the distance between the mineral soil and the litter surface. Litter-layer depth was used as a measure of disturbance intensity. This indirect measure was considered to be more adequate and a between habitats comparable variable, i.e. between grazed grasslands and mown road verges, compared to vegetation height. The number of vascular species was measured in three 5-dm<sup>2</sup> plots randomly placed within populations. Species richness is considered to be a good approximation of habitat quality for species belonging to semi-natural grasslands.

Distances among populations were measured using digital maps in ArcView 3.2. The nearest population distance was either within 100 metres or several kilometres away (Table 1). The measure was included in the statistical analysis to control for the potential influence of isolation on fecundity. All ten localities were, though, highly isolated (>7 kilometres apart). Semi-natural grassland area ranged in size between 0.7 and 13.6 hectares. Habitat area for road verges, usually a few meters wide linear fragments of grassland, was difficult to delineate and measure digitally. Habitat area was, therefore, not included in the statistical analyses.

## Data analysis

In order to compare the relative amounts of variation in fecundity among populations, the coefficient of variation (CV (%)) =  $SD/mean \times 100$  was calculated for the fecundity measures.

We used *t*-test to compare abundance, fecundity and local factors between semi-natural grasslands and road verges. To analyse whether abundance had an influence on fecundity when accounting for local factors and isolation, a forward multiple regression analysis was used. Abundance, soil pH, litter depth, and vascular species richness and isolation were set as independent variables and the fecundity measures as dependent variables. The probability threshold for a variable to be included in the model was  $P = 0.10$ . The influence of abundance on fecundity was analysed with population size and density in separate models (model I with popula-

**Table 1.** The 18 populations of *Euphrasia rostkoviana* ssp. *fennica* investigated in ten localities in either semi-natural grassland or road verge habitats. Populations situated in the same locality share the same number (see Fig. 1). Population size (individual number), mean density ( $n = 3$ ), the mean number of fruits and seeds per fruit per individual, pH ( $n = 2$ ), mean litter-layer depth ( $n = 3$ ), mean number of vascular species ( $n = 3$ ), distance to nearest population (isolation) and the age of the population (local history) are presented. The fecundity measures are based on counts on 20 individuals per population (15 individuals in the smallest population). Standard deviations ( $\pm$  SD) are given in parentheses. The local history data are based on population presence/absence in earlier inventories, i.e. whether the populations were present or not in all the earlier inventories (see Karlsson 1982, and references therein).  $t$ -test was used to compare abundance, fecundity and the local factors between habitat types, the results ( $P$  values) are presented below.

Habitat type	Population size	Population density	Number of fruits	Number of seeds/fruit	pH	Litter-layer (cm)	Number of species	Isolation (m)	Local history (years)
1. Grassland	212	17 (11)	39 (13)	12 (3)	6.92 (0.01)	1.3 (0.2)	23 (3)	13000	15
2. Grassland	950	17 (5)	35 (15)	17 (3)	6.94 (0.04)	1.2 (0.1)	29 (4)	61	102
2. Grassland	175	4 (3)	21 (18)	13 (4)	6.91 (0.03)	1.1 (0.1)	25 (5)	61	102
3. Grassland	78	6 (2)	13 (6)	10 (3)	6.91 (0.01)	0.8 (0.2)	20 (1)	18000	15
4. Grassland	12500	29 (10)	65 (48)	16 (4)	6.91 (0.33)	1.4 (0.3)	19 (1)	16000	60
5. Grassland	2100	69 (5)	55 (42)	17 (3)	5.15 (0.06)	2.7 (0.7)	13 (1)	60	60
5. Grassland	115	23 (6)	47 (28)	14 (3)	5.48 (0.03)	1.5 (0.2)	19 (3)	60	60
7. Grassland	93	23 (4)	18 (9)	14 (2)	6.73 (0.45)	1.5 (0.5)	18 (2)	55	62
7. Grassland	1700	25 (7)	13 (4)	16 (2)	6.31 (0.01)	1.6 (0.4)	18 (2)	55	62
8. Grassland	1420	17 (6)	27 (15)	12 (4)	6.27 (0.37)	1.6 (0.3)	17 (1)	91	15
8. Grassland	595	20 (12)	32 (19)	16 (4)	5.81 (0.19)	1.1 (0.3)	18 (1)	91	15
10. Grassland	2635	17 (9)	30 (14)	12 (2)	6.40 (0.46)	1.7 (0.4)	18 (2)	76	101
6. Road verge	13550	17 (3)	55 (38)	20 (3)	7.05 (0.01)	1.2 (0.8)	13 (1)	9500	60
9. Road verge	28	7 (2)	22 (15)	11 (3)	6.84 (0.26)	2.2 (0.7)	16 (1)	85	5
9. Road verge	1820	26 (9)	50 (30)	18 (2)	6.85 (0.17)	0.4 (0.1)	16 (2)	65	5
9. Road verge	96	7 (2)	29 (19)	11 (2)	6.96 (0.05)	0.5 (0.1)	12 (1)	63	5
9. Road verge	49	7 (4)	7 (2)	7 (2)	6.64 (0.44)	0.4 (0.1)	11 (1)	63	5
10. Road verge	15	7 (3)	21 (16)	11 (3)	6.92 (0.07)	0.7 (0.1)	15 (2)	76	101
$P$	0.259	0.112	0.721	0.321	0.084	0.060	0.006		

tion size as abundance measure, model II with population density as abundance measure). The analyses were based on population mean values (see Table 1). Prior to the analyses, the variables were checked for normality and if necessary log-transformed. The CV values were arcsine-transformed. All analyses were done in Statistica™ 7.0.

## Results

### Study populations and sites

The subspecies was still present in 53% ( $n = 19$ ) of the previously occupied localities. Population sizes varied considerably among populations with a maximum number of 13 550 individuals and minimum number of 15 individuals (Table 1). The average fruit production differed almost 10-fold with a mean number of 32 fruits per individual ( $n = 18$ ,  $SD = 17$ ). The mean number of seeds produced per fruit in populations ranged between 7 and 20. The coefficient of variation (CV) in fruit production ranged between 28% and 85% among the populations, as compared with 12% and 35% for seed production per fruit. The majority of the populations (10 of 18) and localities (6 of 10) were found to be 60 years or older (Table 1).

Road verge and grassland populations did not differ in abundance or fecundity (Table 1). Among the examined local factors, only the number of vascular species differed significantly between road verge and grassland habitats ( $P = 0.006$ ), with more species in grasslands, as expected. Litter-layer depth tended also to differ between habitats. Due to the few differences between habitats, the populations were analysed together.

### Patterns of abundance and fecundity

The two investigated variables associated with abundance, population size and density, were found to be significantly positively correlated (Pearson correlation:  $n = 18$ ,  $r = 0.65$ ,  $P = 0.003$ ). Positive relationships between the individual traits measured in the field were also

found (Table 2). Plant size (height) was positively correlated with the number of flowers produced. Flower number was positively related to both fruit number and seed number per fruit. Furthermore, a positive correlation between the average seed production per fruit and the total number of fruits was found ( $r = 0.73$ ,  $P < 0.001$ ) for the study populations.

For *E. rostkoviana* ssp. *fennica*, both the population size (Fig. 2) and density were positively correlated with fecundity. The number of fruits produced and the mean number of seeds produced per fruit and individual were mainly explained by abundance (Table 3). For instance, 50% of the variation in seed production per fruit was explained by population size. The local factors (pH, litter-layer depth, species richness) and isolation were not significantly related to fecundity. A significant negative relationship was also found between the coefficient of variation in seed production per fruit and population density (Fig. 3). A similar trend was also detected for population size ( $n = 18$ ,  $r = -0.41$ ,  $P = 0.089$ ). No such relationship was detected between the coefficient of variation in fruit production and abundance ( $P > 0.700$ ).

## Discussion

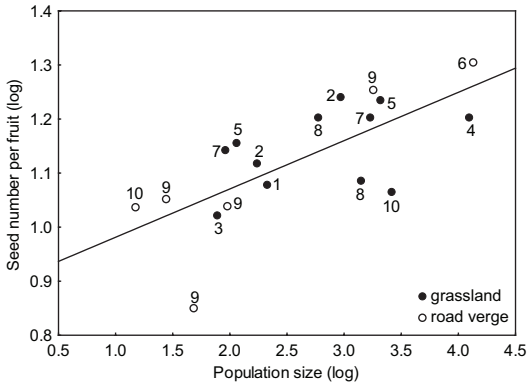
### Study populations and sites

In this study, the rare and declining grassland annual *E. rostkoviana* ssp. *fennica* was found

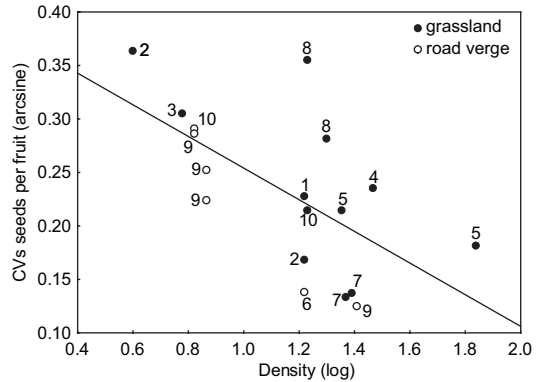
**Table 2.** The relationships between the investigated individual traits in the 18 study populations. In each population 20 individuals (15 in the smallest population) were measured regarding size (height), flower number, fruit number and number of seeds per fruit. Pearson correlation coefficients together with significance levels are presented ( $n = 18$ ,  $P > 0.05$  (ns),  $P < 0.05$  (\*),  $P < 0.01$  (\*\*), and  $P < 0.001$  (\*\*\*)). Number of flowers, number of fruits and seeds per fruit were log-transformed in the analyses.

	Flower number	Fruit number	Seed number per fruit
Size	0.47*	0.05 <sup>ns</sup>	-0.17 <sup>ns</sup>
Flower number		0.71***	0.51*
Fruit number			0.73***





**Fig. 2.** The positive relationship between mean seed number per fruit and population size in *Euphrasia rostkoviana* ssp. *fennica* ( $n = 18$ ,  $r = 0.71$ ,  $P = 0.001$ ). Locality numbers (1–10) are given (see Fig. 1).



**Fig. 3.** The negative relationship between the coefficient of variation (CV) in seed number per fruit and population density in *Euphrasia rostkoviana* ssp. *fennica* ( $n = 18$ ,  $r = -0.62$ ,  $P = 0.006$ ). Locality numbers (1–10) are given.

in ten of the 19 investigated localities, and half of the populations were located in road verges. The majority of the extinct localities were abandoned, and the taxon has therefore most likely disappeared from these sites due to ceased management (e.g. Karlsson 1982, Aronsson 1999). Interestingly, the largest population in Sweden today is situated in a road verge in the study area. This population was examined in the summer of 2004 and was found to consist of around 1000 individuals, as compared with 13 550 individuals we found in 2005. Evidently, this population has recently been increasing rapidly in size and the road verge habitat in this example has probably been more than just a “rescue site” for the population. The investigated subspecies seems thus to be able to recruit successfully in moderately grazed grasslands as well as along yearly mown gravelled road verges resembling semi-natural grasslands.

### Patterns of abundance and fecundity

In this study, larger populations of *E. rostkoviana* ssp. *fennica* were also denser. Abundance of conspecifics was the only factor that was significantly positively correlated with fecundity, which thus demonstrates the presence of an Allee effect in the investigated taxon. The examined local factors, or isolation, could not explain any variability in fecundity. In accordance with many other studies concerned with fragmentation effects on reproductive output (e.g. Jennersten 1988, Ågren 1996, Kunin 1997, Morgan 1998, Kolb 2005), we found a pattern of decreased fecundity measured as average seed production per fruit and total fruit production per individual in small and sparse populations of *E. rostkoviana* ssp. *fennica*. The causality behind reduced fecundity varies between studies, but generally disrupted plant–pollinator interactions are supposed to be of vital importance, especially

**Table 3.** Relationship between fecundity and abundance (population size and density), local factors (soil pH, litter depth and vascular species richness) and isolation as determined by multiple linear regression (forward method). Only the final models are presented (see Material and methods for details of model selection). The variables were log-transformed in the analyses.

Response variable	Predictor variables	$\beta$ (SE)	$t_{16}$	$P$	$r^2$
Fruit number	(I) Population size	0.57 (0.21)	2.79	0.013	0.33
	(II) Population density	0.57 (0.21)	2.75	0.014	0.32
Seed number per fruit	(I) Population size	0.71 (0.18)	4.01	0.001	0.50
	(II) Population density	0.65 (0.19)	3.44	0.003	0.42

in self-incompatible species. The present distribution of the study species is highly fragmented and we suspect that dispersal among populations may be too limited.

Several studies have shown that the spatial pattern of individuals in combination with tolerance to self-pollen may be vital in determining the reproductive output in plant populations (e.g. Kunin 1997, Mustajärvi *et al.* 2001). Contrary to earlier studies, Mustajärvi *et al.* (2001) recorded higher reproductive success in sparse populations of the self-compatible sticky catchfly (*Viscaria vulgaris*) as compared with that in denser populations. The proposed explanation was that individuals in sparse populations, as compared with those in denser populations, suffered less from resource competition. This interesting pattern may hold for self-compatible plants species, which to some degree can tolerate the deteriorating effects of inbreeding, but is probably less important in self-incompatible species, which are highly dependent on animal vectors for successful reproduction (Mustajärvi *et al.* 2001). In this study, fecundity was not reduced in denser populations, which imply that the influence of negative density dependence on reproduction in *E. rostkoviana ssp. fennica* is most likely weak (e.g. Stephens *et al.* 1999)

The increased probability of inbreeding in isolated small populations could thus contribute to the overall reduction in fecundity, because the chances of matings between close relatives increase. Generally, a strong positive association between self-pollination and the annual life-form exists in plants (Aarssen 2000). *Euphrasia rostkoviana ssp. fennica* is self-compatible (Karlsson 1982), but the female and male reproductive organs are separated both temporally (dichogamy) and spatially (herkogamy), making autogamous or geitonogamous pollen transfer a less possible solution when outcrossing fails. The degree of Allee effect, i.e. increasing fecundity with increasing abundance of conspecifics, is expected to depend on the specific mating system of the species. Due to the morphological adaptations for outcrossing in *E. rostkoviana ssp. fennica*, Allee effects are surely of importance for this endangered species.

The significant negative association between density and the coefficient of variation in seed

production per fruit was quite surprising to find. The greater variability in seed number per fruit, which also corresponds to a lower mean number of seeds per fruit, for individuals in small and sparse populations may be a result of inbreeding depression caused by increased levels of selfing. Studies have shown that inbred plants often are smaller, and produce fewer flowers and seeds (e.g. Fischer & Matthies 1997, Hooftman *et al.* 2003). In this study, the capacity to produce many fruits and seeds per fruit was positively related to (besides abundance of conspecifics) the flower production of individuals, which in turn was positively related to the size of individuals within populations. The reduced fecundity in small and sparse population of *E. rostkoviana ssp. fennica* may further imply a lower dispersal capacity since seed production *per se*, is an important factor shaping dispersal potential (e.g. Jakobsson & Eriksson 2000).

## Conclusions

Fragmentation and isolation of plant populations are thought to affect demographic processes such as seed production and cause reduction in fitness (Young *et al.* 1996, Morgan 1999). The presence of an Allee effect in the investigated taxon predisposes small populations of *E. rostkoviana ssp. fennica* to be highly uncertain and ephemeral in occurrence. Furthermore, *Euphrasia* species, in general, have a problem with recruiting in mature vegetation and in spots with a thick litter-layer (Karlsson 1982). Considering the ecology of this rare annual subspecies, small populations (a few hundred individuals) should be expected to experience a high local risk of extinction and should therefore be managed with increased prudence.

Management of road verges adjacent to grassland populations, due to the ability of utilizing road verges, could be important in promoting the dispersal between isolated grasslands. Likewise, an improved status of grasslands along road verges with present populations could be fruitful. It should though be stated that despite the overall high risk of extinction experienced by the investigated taxon, many populations were still present since the last inventory (i.e. after



several decades) and some populations had also increased in size.

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