



Inbreeding and soil conditions affect dispersal and components of performance of two plant species in fragmented landscapes

Carolin Mix^{a,*}, F. Xavier Picó^b, Jan M. van Groenendael^a, N. Joop Ouborg^a

^aDepartment of Ecology, Radboud University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

^bCentre for Ecological Research and Forestry Applications (CREAF), Faculty of Sciences, Autonomous University of Barcelona, 08193 Bellaterra (Barcelona), Spain

Received 26 April 2005; accepted 28 April 2005

KEYWORDS

Crossing experiment;
Genetic erosion;
Habitat
fragmentation;
Inbreeding
depression;
Lifespan;
Mating system;
Population size;
Seed dispersal traits;
Soil deterioration

Summary

During habitat fragmentation, plant populations become smaller and more isolated from each other, resulting in increasing inbreeding rates within populations. Furthermore, fragmentation is often accompanied by a progressive deterioration of soil conditions. Overall, high inbreeding rates and poor soil conditions decrease plant performance and so increase the probability of extinction of fragmented plant populations. The goal of this study was to investigate the effects of inbreeding and soil acidification on seed and offspring traits of *Succisa pratensis* and *Hypochaeris radicata*, two plant species differing in mating system, lifespan and dispersal ability. For each species, plants from four populations of different sizes were hand-pollinated. The selfed and outcrossed progeny were grown at two soil pH levels. Overall, results showed that the dispersal potential of *H. radicata* was reduced by selfing, indicating that dispersal capacity is not independent from the genetic erosion process. Variation among seed families and its interactions with pollination treatments indicate that dispersal capacity may have a genetic basis. The performance of both species decreased sharply as soil conditions became more acidic, but inbreeding did not aggravate the process. These results suggest that *S. pratensis* and *H. radicata* populations may decline in the long term; however, family level variation suggests a potential for adaptation to new conditions.

© 2005 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Zusammenfassung

Habitatfragmentierung resultiert in kleinen, voneinander isolierten Populationen, die ein höheres Maß an Inzucht aufweisen können. Desweiteren findet neben der

*Corresponding author. Tel.: +31 24 365 3047; fax: +31 24 365 2134.
E-mail address: c.mix@science.ru.nl (C. Mix).

Fragmentierung oft eine Verschlechterung der Bodenverhältnisse statt. Im Allgemeinen wird angenommen, dass hohe Inzuchtraten und verschlechterte Bodenverhältnisse die Vitalität von Pflanzen reduzieren und damit das Aussterben der fragmentierten Pflanzenpopulationen fördert. Das Ziel unserer Forschung war es, die Effekte von Inzucht und Bodenversauerung an Sameneigenschaften und Eigenschaften der Nachkommenschaft von *Succisa pratensis* und *Hypochaeris radicata* zu untersuchen. Beide Pflanzenarten unterscheiden sich in ihren Fortpflanzungsstrategien, der Langlebigkeit und der Samenausbreitung. Wir führten Handbestäubungen an Pflanzen von vier Populationen unterschiedlicher Populationsgrößen durch. Die Nachkommenschaft der fremd- und selbstbestäubten Pflanzen wurde in Böden mit unterschiedlichem pH aufgezogen. Die Resultate zeigen, dass das Ausbreitungsvermögen von *H. radicata* nach Selbstbestäubung reduziert wurde, was darauf hinweist, dass Samenverbreitung nicht unabhängig vom Prozess der genetischen Erosion ist. Variationen zwischen Pflanzenfamilien und Wechselwirkungen zwischen Familien und Bestäubungsbehandlung deuten darauf, dass die Variation im Samenausbreitungsvermögen einen genetischen Ursprung hat. Die Vitalität der Pflanzen beider Arten wurde stark reduziert, wenn die Bodenverhältnisse saurer waren, obwohl Inzucht diesen Prozess nicht verschlechterte. Diese Resultate deuten darauf hin, dass beide Arten in Zukunft zurückgehen werden. Die Variation auf dem Familienniveau dagegen zeigt die Anwesenheit eines Vermögens sich an neue Umweltverhältnisse anzupassen.

© 2005 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Introduction

Habitat fragmentation and deterioration are major threats to the persistence of plant populations. In the process of habitat fragmentation, plant populations become smaller and more isolated from each other. As a result, the number of breeding individuals within a population and the level of gene flow between populations decrease, leading to increasing inbreeding and genetic drift (Dudash & Fenster, 2000; Ellstrand & Elam, 1993). High inbreeding rates increase the proportion of homozygous individuals that may be affected by inbreeding depression (i.e. reduction of fitness of selfed compared to outcrossed progeny), mainly caused by the expression of deleterious recessive alleles (Charlesworth & Charlesworth, 1999). Hence, fragmented plant populations tend to exhibit reduced fitness due to inbreeding depression, and the ability of plants in fragmented habitats to cope with this problem may be important to avoid local extinction (Young & Clarke, 2000). Outbreeding between populations often improves the fitness of the progeny (i.e. heterosis), but may lead to reduced fitness (i.e. outbreeding depression; Waser, Price, & Shaw, 2000) by disrupting locally adapted populations (Hufford & Mazer, 2003) or co-adapted gene complexes or other favourable epistatic relationships (Lynch, 1991).

In addition to reduced fitness, genetic diversity tends to decrease (i.e. genetic erosion) in fragmented populations (Dudash & Fenster, 2000). Fragmented populations might be genetically "res-

cued" by immigrants from other populations (Brown & Kodric-Brown, 1977; Richards, 2000), reducing inbreeding depression and increasing the level of genetic variation (Ingvarsson, 2001). So far, dispersal has been considered to be independent from the genetic erosion process. However, results of several studies indicate that variation in dispersal may have a genetic basis (Clay, 1982; Olivieri, Swann, & Goyon, 1983; Schmitt, Ehrhardt, & Schwartz, 1985), although there is as yet insufficient proof of this. It may be hypothesized that species-specific dispersal traits, such as seed size and inflorescence height, might become affected by inbreeding.

Habitat fragmentation often co-occurs with changes in habitat quality due to factors such as soil acidification, eutrophication and desiccation. Deterioration of the habitat has been shown to reduce the individual fitness of plants (Vergeer, Rengelink, Ouborg, & Roelofs, 2003). However, the combination of inbreeding and soil acidification has been largely neglected. Therefore, a comparison of the growth and reproduction of selfed and outcrossed progeny at different soil pH levels might be useful to quantify the response of a plant species to inbreeding.

The goal of this study was to determine the effects of inbreeding and outbreeding on dispersal-related traits and to compare the growth and reproduction of selfed and outcrossed progeny of two plant species, differing in terms of mating system, lifespan and dispersal strategy, at two soil pH levels. *Succisa pratensis* is a mixed-mating, long-lived perennial with no specific dispersal

mechanism, and *Hypochaeris radicata* is a mainly outcrossing, short-lived perennial with plumed seeds, allowing long-distance dispersal by wind (Soons, Heil, Nathan, & Katul, 2004). Both species coexist in the Netherlands in nutrient-poor grasslands, which have become highly fragmented during the past 100 years (Soons, 2003). Experimental hand pollinations were used to produce selfed and outcrossed progeny of plants from four populations differing in population size. Selfed and outcrossed progeny were raised in the greenhouse at two contrasting soil pH levels that plants can encounter in the field. We discuss the implications of inbreeding and outbreeding for the survival of *S. pratensis* and *H. radicata* populations in a fragmented habitat.

Materials and methods

Species and area studied

S. pratensis Moench (Dipsacaceae) occurs in nutrient-poor, moist grasslands. The rosettes, which reach a diameter of approximately 15 cm, can produce 1–6 flower stalks with heights of 20–100 cm. The species can also generate lateral side rosettes at the ends of its stolons. Genets can live for more than 25 years (Hoofman, Van Kleunen, & Diemer, 2003). Flowering occurs during August and September. Flower heads contain 60–100 single florets. The hermaphroditic, protandric flowers are mainly pollinated by insects. Short-distance seed dispersal by wind may occur over distances up to 4 m (Soons et al., 2004). Germination takes place in spring.

H. radicata L. (Asteraceae) also occurs in nutrient-poor grasslands, though the species can

also be found in open vegetation sites and in forest margins. *H. radicata* can reproduce clonally. Genets live for about 3 years. The rosettes, which reach a diameter of approximately 20 cm, produce 3–12 branched flower stalks with heights of 8–30 cm. The flowering period lasts from May to September, with two flowering peaks. The hermaphroditic, protandric flowers are insect-pollinated. The species is considered to be an obligate outbreeder and mainly self-incompatible (Fryxell, 1957), although Hagerup (1954) and Grime, Hodgson, and Hunt (1988) reported that self-pollination may occur. The species produces single-seeded fruits that bear a pappus and are adapted to long-distance dispersal by wind over distances up to 400 m (Soons et al., 2004). The seeds have no specific germination requirements.

We selected four populations per species, differing in size and distance to neighbouring populations (Table 1). While *H. radicata* has a much wider distribution than *S. pratensis*, both species are present in nature reserves and roadside ditches, along railway tracks and waterways. Distances to the nearest neighbouring population ranged from 0.38 to 4.25 km and from 2 to 125 m for *S. pratensis* and *H. radicata*, respectively. The *H. radicata* population HM is a subsample of a continuum of that species (Table 1).

Crossing experiment

We collected seeds of *S. pratensis* during summer 1998, from 15 randomly sampled plants (hereafter families) for each population studied. The seeds of one plant are hereafter referred to as 'F1-families'. Seeds were kept in paper bags at room temperature until spring 1999, when they were sown in 10 cm × 10 cm pots. Four weeks after germination,

Table 1. Characteristics of the *Succisa pratensis* and *Hypochaeris radicata* populations studied. Coordinates refer to latitude and longitude. The population size (i.e. the number of flowering individuals) is indicated by N_e

Population	Code	Coordinates	N_e	Distance (km)	No. of plants
<i>S. pratensis</i>					
Kooierlaan	SKL	52° 05' N, 6° 31' E	50	4.250	15
Koolmansdijk	SKM	52° 01' N, 6° 33' E	1500	1.200	15
Konijnendijk	SKD	52° 02' N, 6° 26' E	15,000	0.375	15
Havelte	SH	52° 48' N, 6° 14' E	25,000	1.300	15
<i>H. radicata</i>					
Allemanskampje	HA	52° 04' N, 5° 34' E	100	0.010	8
Havelte	HH	52° 48' N, 6° 14' E	1000	0.020	14
Bennekom	HB	52° 00' N, 5° 35' E	3500	0.125	13
Molenweg	HM	52° 01' N, 6° 34' E	30,000	0.002	14

Distances to neighbouring populations and numbers of plants used in the experiment are also given.

two seedlings per family (of the 15 families per population) were individually transplanted into 10 cm × 10 cm pots filled with standard soil mixture. Plants were grown in a greenhouse without climate regulation and watered regularly until summer 2000, when the crossing experiment in the greenhouse started. The first five flower heads per family were chosen to create five different cross lines. All pollinations were carried out by hand pollinating on one of the two siblings per family. A selfing line (INBRED1) was obtained, always using the first flower with its own pollen. A biparentally inbred line (INBRED2) was created using the other siblings as pollen donors. Three outcrossing lines were created for each family by performing a within-population cross (OUTCROSS1) with a pollen mixture of 3 to 4 other families of the same population. In addition, two between-population crosses were performed, using a pollen mixture from 3 to 4 other families of one population (other than the neighbouring population or the study populations) located at distances between 1 and 20 km to the populations of study (OUTCROSS2) and one population (again other than the neighbouring population or the study populations) located at distances between 30 and 40 km (OUTCROSS3) from each population of study. Recipient heads were emasculated prior to hand pollination. Heads were pollinated by brushing the pollen donor head over the recipient head. We bagged all hand-pollinated flower heads during the treatment to avoid uncontrolled pollinations, and left them bagged until seed harvest.

Seeds of *H. radicata* were collected in the summer of 1999, from eight to 14 randomly sampled plants (hereafter families) for each population studied. The seeds of one plant are hereafter referred to as 'F1-families'. Seeds were kept in paper bags at room temperature until the spring of 2000, when they were sown in 10 cm × 10 cm pots filled with standard soil mixture. Two weeks after germination, two seedlings per family were individually transplanted into 10 cm × 10 cm pots and raised in the greenhouse under the same conditions as *S. pratensis*. Emasculatation was not feasible in *H. radicata*, as is the case for many other composites. We used the same pollination treatments and procedures as for *S. pratensis*.

Seed and plant measurements

In January 2001, we measured seed length for both species, and pappus diameter for *H. radicata*. Measurements were conducted using the OPTIMAS 6.5 image analysis software (Media Cybernetics,

1999) on eight randomly chosen filled fruits per family and pollination treatment (about 4800 seeds overall). This software allows simultaneous and accurate measurements of different seed traits by analyzing the image of the seed taken by a camera that is attached to a microscope (Mix, Picó, & Ouborg, 2003). Seed mass was estimated by linear regression methods between the seed mass and seed length obtained by the image analysis software for a subsample of seeds from the OUTCROSS1 pollination treatment ($R^2 = 0.70$, $F_{1,160} = 369.6$, $P < 0.001$, for *S. pratensis*; $R^2 = 0.74$, $F_{1,28} = 61.1$, $P < 0.001$, for *H. radicata*). Terminal velocity (V_t), the inverse of the dispersal potential, was measured according to Soons and Heil (2002), who parameterized the following terminal velocity functions for *S. pratensis* and *H. radicata*, respectively:

$$V_t = 0.64 + (1.17 \times (\text{seed mass})^{1/2}),$$

$$R^2 = 0.26 (P < 0.001), \quad (1)$$

$$V_t = 5.93 \times (\text{seed mass/pappus diameter})^{1/2},$$

$$R^2 = 0.59 (P < 0.001). \quad (2)$$

In April 2001, *S. pratensis* and *H. radicata* seeds were placed in small pots for germination. In May/June 2001, we transplanted up to five seedlings per F1-family per population per pollination treatment and per species into 10 cm × 10 cm pots filled with two different soil types differing in pH (up to five seedlings per soil type, about 2000 seedlings overall), to test the effects of pollination treatment on plant traits of offspring under different environmental conditions. The pH values were 4.2, representing stressful acidified conditions, and 5.5, which is close to conditions commonly found in the field for both species (Grime et al., 1988). The soil was obtained from a 40 to 60 cm soil layer at the De Hamert nature reserve (owned and managed by Limburgs Landschap, The Netherlands), which had a pH of 4.2. In order to raise the pH to 5.5, we added 3.2 g/dm³ Dolokal (Ankerpoort Inc., The Netherlands) containing 95% CaCO₃ and 5% MgCO₃. Twice a week, plants were watered with rain water. During the experiment, we measured the pH of the soil four times, and found that it changed only slightly during this period (± 0.05).

In August 2002, we determined the total biomass of three *S. pratensis* plants per family per population per pollination treatment and per soil pH level after drying them for 24 h at 74 °C. For *H. radicata*, we recorded the leaf width of the largest leaf and the number of leaves of all plants, and estimated their total biomass using an equation obtained

from linear regression between dry mass and the product of the width of the largest leaf and the number of leaves of a subsample of plants from the OUTCROSS1 pollination treatment ($R^2 = 0.71$, $F_{1,18} = 41.8$, $P < 0.001$). The length of the flower stalks and the number of flower heads per stalk were counted after flowering in June and August of 2002 for *H. radicata* and *S. pratensis*, respectively.

Statistical analysis

The effects of pollination treatment (fixed factor), population (random factor) and family nested within population (random factor) on seed length, dispersal attributes and terminal velocity were analysed with separate two-way ANOVAs for each species. We analysed the effects of pollination treatment (fixed factor), soil pH (fixed factor), population (random factor) and family nested within population (random factor) on total biomass, the length of flower stalks, and the number of flower heads per stalk, using separate three-way ANOVAs for each species. A Tukey posthoc test was used to determine significant differences between pollination treatments. Dependent variables were log (terminal velocity) or square (seed length) transformed to meet ANOVA assumptions.

Linear regression analysis was used to study the effects of population size on dispersal and plant performance parameters. Population size was log-transformed in these analyses.

Results

Succisa pratensis

Seed dispersal characteristics

None of the seed dispersal characteristics of the F1-families was significantly affected by the pollination treatments (Table 2). Seed length differed significantly between populations (Table 2), being largest in the smallest population (Table 3), although there was no significant relationship with population size. The pollination treatment \times population interaction was significant for terminal velocity (Table 2), indicating that populations responded differently. Both seed length and terminal velocity differed significantly between families within populations, and the effects of pollination treatment on these traits varied significantly between families (Table 2). Population size was not related to any of the seed size and dispersal characteristics.

Offspring performance

Pollination treatment had no effect on any of the traits measured in the F1-families of offspring (Table 4). Total biomass, the length of the flower stalks and the number of flower heads per stalk were significantly larger for plants in the high soil pH condition (Table 4, Fig. 2). Total biomass and the number of flower heads per stalk varied significantly between populations (Table 4). The length of the flower stalks of the F1-families varied

Table 2. Two-way ANOVAs testing for the effects of pollination treatment (INBRED1, INBRED2, OUTCROSS1, OUTCROSS2 and OUTCROSS3), population (four populations), family nested within population, and interacting effects on dispersal traits of *S. pratensis* and *H. radicata*

Factor	d.f.	d.f. Error term	Seed length			Pappus length			Terminal velocity		
			MS	F	P	MS	F	P	MS	F	P
<i>S. pratensis</i>											
Treatment (T)	4	12	0.027	2.62	0.08	–	–	–	0.226	1.18	0.37
Population (P)	3	56	0.199	4.12	0.01	–	–	–	1.220	1.85	0.15
Family (F)	56	2017	0.048	7.74	<0.001	–	–	–	0.659	6.82	<0.001
T \times P	12	213	0.010	1.63	0.86	–	–	–	0.192	1.99	0.03
T \times F	213	2017	0.006	12.77	<0.001	–	–	–	0.097	13.40	<0.001
Error	2017		<0.001						0.007		
<i>H. radicata</i>											
Treatment (T)	4	11	0.037	7.86	0.003	0.295	2.57	0.1	1.978	3.52	0.02
Population (P)	3	51	0.248	4.76	0.005	0.485	0.89	0.46	3.021	2.99	0.04
Family (F)	51	1269	0.052	3.42	<0.001	0.548	4.17	<0.001	1.012	2.10	<0.001
T \times P	11	115	0.005	0.31	0.99	0.115	0.87	0.58	0.562	1.66	0.09
T \times F	115	1269	0.015	20.38	<0.001	0.132	3.37	<0.001	0.339	1.92	<0.001
Error	1269		0.01			0.039			0.177		

Table 3. Means (\pm SE) of seed size, dispersal and offspring performance traits of the *Succisa pratensis* and *Hypochaeris radicata* populations studied. For population codes see Table 1

Population	Seed length (mm)	Pappus length (mm)	Terminal velocity (m/s)	Total biomass (g)	Stalk length (cm)	No. of heads per stalk
<i>S. pratensis</i>						
SKL	4.12 \pm 0.31	—	2.02 \pm 0.02	7.53 \pm 0.30	60.93 \pm 0.97	3.31 \pm 0.07
SKM	3.71 \pm 0.22	—	1.79 \pm 0.02	7.48 \pm 0.33	67.15 \pm 1.26	3.33 \pm 0.10
SKD	3.90 \pm 0.31	—	1.78 \pm 0.01	8.36 \pm 0.32	73.67 \pm 0.85	3.92 \pm 0.09
SH	3.60 \pm 0.23	—	1.75 \pm 0.02	7.00 \pm 0.22	72.46 \pm 0.86	3.03 \pm 0.06
<i>H. radicata</i>						
HA	4.5 \pm 0.04	6.6 \pm 0.12	0.54 \pm 0.02	2.16 \pm 0.10	36.16 \pm 0.50	3.44 \pm 0.10
HH	4.4 \pm 0.03	6.9 \pm 0.11	0.63 \pm 0.02	2.14 \pm 0.10	33.12 \pm 0.47	2.50 \pm 0.06
HB	4.8 \pm 0.04	7.4 \pm 0.14	0.67 \pm 0.02	2.40 \pm 0.11	34.58 \pm 0.45	2.40 \pm 0.06
HM	5.0 \pm 0.04	6.5 \pm 0.09	0.71 \pm 0.03	2.51 \pm 0.12	32.83 \pm 0.50	2.46 \pm 0.08

Table 4. Three-way ANOVAs testing for the effects of pollination treatment (INBRED1, INBRED2, OUTCROSS1, OUTCROSS2 and OUTCROSS3), soil condition (pH 4.2 and 5.5), population (four populations) and family nested within population on total biomass, stalk length and number of flower heads per stalk of *Succisa pratensis* and *Hypochaeris radicata*

Factor	d.f.	d.f. Error term	Biomass			Stalk length			No. heads		
			MS	F	P	MS	F	P	MS	F	P
<i>S. pratensis</i>											
Treatment (T)	4	11	15.17	3.21	0.06	655.10	2.77	0.08	0.06	0.06	0.99
Soil (S)	1	3	2502.14	45.92	0.007	16,881.81	18.97	0.02	49.39	12.57	0.04
Population (P)	3	25	71.70	4.15	0.02	6670.66	7.10	0.001	29.51	7.56	0.001
Family (F)	25	df _{error}	17.29	0.91	0.58	939.39	3.30	0.003	3.90	1.44	0.14
T \times S	4	11	12.35	1.27	0.34	88.57	0.40	0.80	1.28	1.42	0.29
T \times P	11	78	4.72	0.26	0.99	236.27	0.92	0.53	1.06	0.42	0.94
T \times F	78	df _{error}	18.19	1.48	0.04	256.93	1.19	0.22	2.54	2.06	0.001
S \times P	3	25	55.36	4.21	0.02	889.77	3.61	0.03	3.93	2.65	0.07
S \times F	25	df _{error}	13.15	1.07	0.39	246.27	1.15	0.31	1.49	1.19	0.27
Error (d.f.)			11.59 (527)			187.49 (822)			1.55 (822)		
<i>H. radicata</i>											
Treatment (T)	4	12	7.31	1.08	0.40	180.21	0.76	0.57	5.01	1.98	0.16
Soil (S)	1	3	62.07	11.94	0.04	2643.33	54.36	0.005	5.19	17.9	0.02
Population (P)	3	28	5.21	0.62	0.61	892.01	3.38	0.03	55.72	8.68	<0.001
Family (F)	28	df _{error}	8.39	2.07	0.03	263.27	2.25	0.006	6.42	6.35	0.003
T \times S	4	12	3.11	0.65	0.64	24.43	0.43	0.78	0.37	0.13	0.97
T \times P	12	81	6.74	1.70	0.08	238.66	2.28	0.02	2.54	1.20	0.30
T \times F	81	df _{error}	3.96	1.54	0.03	104.68	2.30	<0.001	2.12	1.20	0.20
S \times P	3	28	5.19	1.94	0.15	48.62	0.84	0.48	0.29	0.45	0.72
S \times F	28	df _{error}	2.67	1.04	0.43	57.68	1.27	0.28	0.64	0.36	0.10
Error (d.f.)			3.07 (913)			62.48 (973)			1.58 (973)		

Three-way interactions were not significant and are not shown.

significantly between populations and families (Table 4) and was positively correlated with population size ($R^2 = 0.97$, $F_{1,3} = 68.3$, $P < 0.05$) (Table 3, Fig. 3). Population SKD exhibited the highest values of total biomass, length of flower stalks and number of flower heads per stalk (Table 3).

The soil pH \times population interaction was significant for total biomass and the length of the flower stalks, indicating that the soil pH effect was larger for large populations (results not shown). The pollination treatment \times family interaction was significant for total biomass and the number of flower

heads per stalk (Table 4). There was no relationship with population size.

Hypochaeris radicata

Seed dispersal characteristics

Pollination treatment significantly affected seed length and terminal velocity (Table 2) of the F1-families. Seed length and terminal velocity were significantly larger for INBRED1 progeny than for the rest of the pollination treatments (Fig. 1). Variation among populations was significant for both variables, which tended to decrease with decreasing population size (Table 3). The only significant relationship with population size was found for terminal velocity ($R^2 = 0.96$, $F_{1,3} = 45.1$, $P < 0.05$), showing that the smallest population (HA)

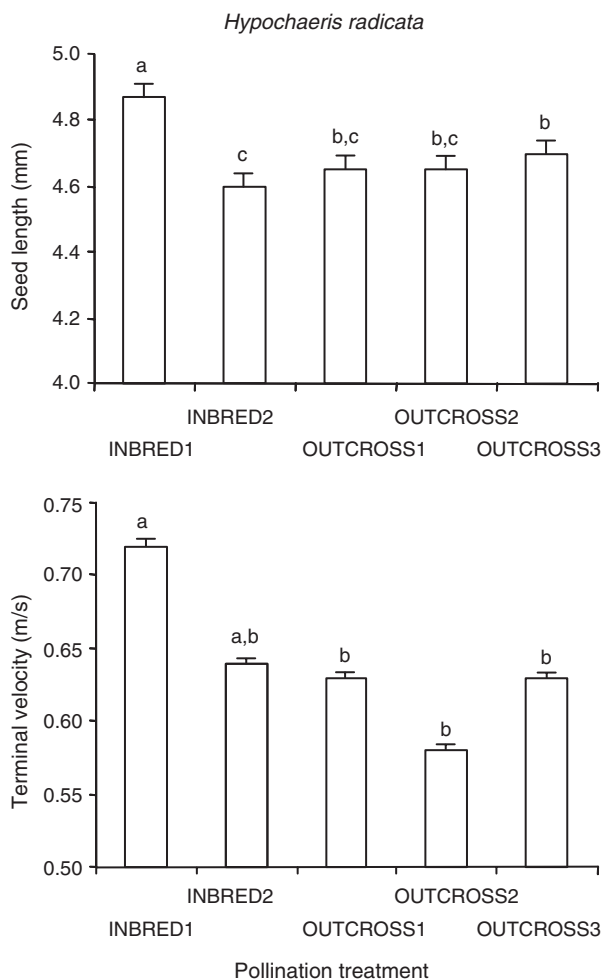


Figure 1. Mean (\pm SE) seed size and dispersal traits for each pollination treatment for *Hypochaeris radicata*. Different letters at top of bars indicate significant differences between means (Tukey's posthoc test, $P < 0.05$). Pollination effects were not significant for *Succisa pratensis*.

exhibited the lowest terminal velocity (Fig. 3). Seed length, pappus length, and terminal velocity varied significantly between families within populations, and the pollination treatment \times family interaction was also significant for all three variables (Table 2).

Offspring performance

None of the performance traits of F1-families of offspring varied significantly between pollination treatments (Table 4). Just as in *S. pratensis*, biomass, stalk length, and the number of flower heads per stalk were significantly larger in soils with higher pH (Table 4, Fig. 2). The length of the flower stalks and the number of heads per stalk varied significantly between populations (Table 4). The length of the flower stalks tended to decrease with increasing population size. The number of heads per stalk was significantly larger in the HA population, which had the smallest size (Table 3), although there was no significant overall relationship with population size. The pollination treatment by population interaction was significant for the length of the flower stalks, but was not related to population size, whereas the pollination treatment \times family interaction was significant for total biomass and the length of the flower stalks (Table 4).

Discussion

Effects of inbreeding on dispersal-related traits

The effects of pollination treatment on dispersal-related traits differed between the two plant species. Inbreeding did not affect any of the traits measured for the long-living *S. pratensis*, while the short-living *H. radicata* clearly showed an increase in seed length and consequently in terminal velocity under inbreeding. These results are in agreement with the prediction that inbreeding might be delayed in long-lived plant species (Byers & Waller, 1999).

A common pattern shared by both species was the significant family effect and the significant pollination treatment \times family interaction for most of the traits, indicating the potential of the populations to adapt to new conditions. Family level variation in inbreeding levels is a common finding in experimental hand-pollination experiments, which can be accounted for by a difference in genetic load between individuals of the population as a result of different histories of inbreeding

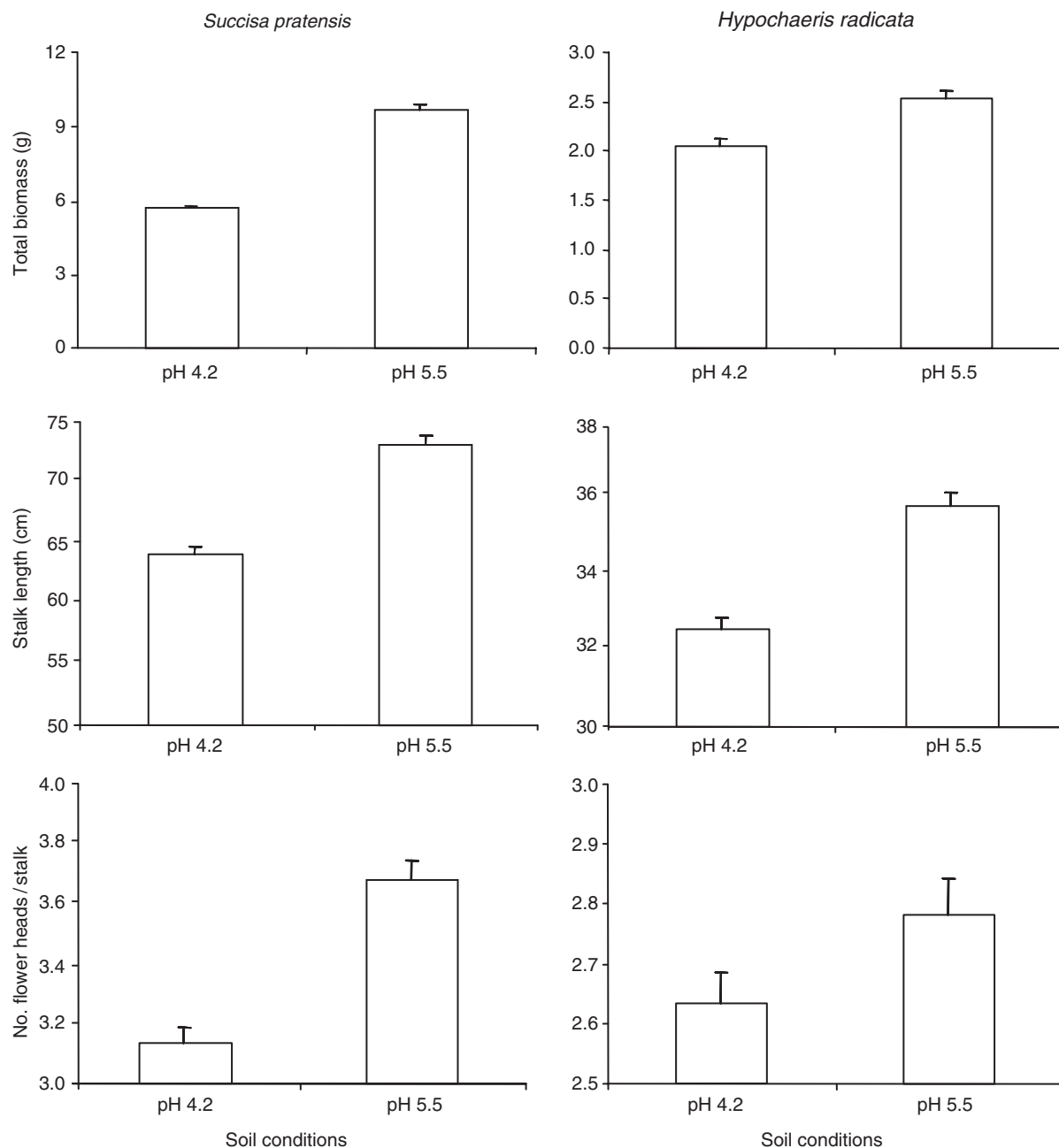


Figure 2. Mean (\pm SE) plant traits for each soil type for *Succisa pratensis* and *Hypochaeris radicata*. For statistical results see Table 4.

(Koelewijn, 1998; Picó, Ouborg, & van Groenendael, 2004; Schultz & Willis, 1995). Family based variation in seed dispersal characteristics might be an indication that dispersal indeed has a genetic basis.

Seed length of *H. radicata* increased under inbreeding. Picó et al. (2004) found that inbred plants of *H. radicata* showed strongly reduced seed set in two populations (one of them being population HA used in this study), which has been suggested to be the consequence of the partly

self-incompatible mating system (Luijten, Kéry, Oostermeijer, & den Nijs, 2002; Picó et al., 2004). These results may indicate a trade-off between seed set and seed size (Crawley, 1997), as inbreeding results in fewer but larger seeds due to allocation of maternal resources to fewer seeds (Picó et al., 2004; Roach & Wulff, 1987). This relationship has, however, not been consistently reported (Lienert & Fischer, 2004).

Terminal velocity in *H. radicata* is determined by seed size and pappus diameter (Soons & Heil,

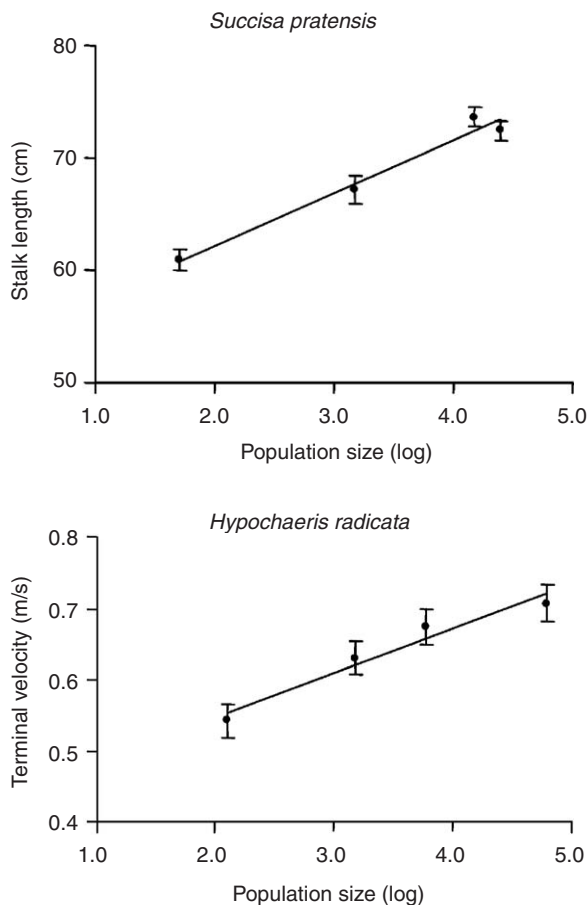


Figure 3. Relationship between population size and mean (\pm SE) stalk length for *Succisa pratensis*, and population size and mean (\pm SE) terminal velocity for *Hypochaeris radicata*, based on four populations per species. Statistical results in Table 4.

2002). Given that selfed *H. radicata* seeds were larger than outcrossed seeds, and that pappus size did not vary between pollination treatments, selfed seeds had a lower dispersal potential (higher terminal velocity) than outcrossed seeds. Thus, selfing in *H. radicata* negatively affected dispersal by increasing seed size, indicating that dispersal is an integral part of the genetic erosion process. If it were only inbreeding which acts on our small populations, the populations should exhibit reduced dispersal ability. However, we found that the smallest population (HA) had the lowest terminal velocity (i.e. highest dispersal ability). From this result we conclude that inbreeding may not be of significance in these populations and that genetic drift may have caused the differences in dispersal ability between populations of different sizes, although maternal effects cannot be excluded (Picó et al., 2004). However, we must emphasize that seeds comprise a complex of different genetic entities (Shaw & Waser, 1994),

increasing the difficulty of relating effects directly to seed traits.

Given the fact that interpopulation distances were small enough to be bridged by *H. radicata* seed migration (since maximum distances to neighbouring populations in our study were about 125 m, while seed dispersal distances are up to 400 m according to Soons et al., 2004) we do not think selection against dispersal due to isolation (Cody & Overton, 1996) or in a metapopulation context (Olivieri, Michalakis, & Gouyon, 1995) occurs. This is also suggested by the results of a molecular study on populations of *H. radicata*, which showed that high levels of seed- and pollen mediated gene flow between the populations are common (Mix et al., unpubl. manuscript), indicating that extended migration may undermine the selection process against dispersal.

Another important parameter facilitating seed dispersal is the length of the flower stalks (i.e. the height at which seeds are released), as taller stalks have greater chances to disperse seeds further away from the mother plant (Soons et al., 2004). Although stalk length in *S. pratensis* was not affected by inbreeding, plants from smaller populations exhibited significantly shorter flower stalks than plants from bigger populations, which might be due to genetic drift. Therefore, smaller populations are expected to show reduced seed dispersal distances, assuming equal wind speed and vegetation height. However, since seeds of *S. pratensis* are not dispersed by wind beyond 4 m (Soons et al., 2004), it is questionable whether a reduction in stalk length would seriously affect the seed dispersal of this species.

Effects of inbreeding and soil acidification on offspring performance

Inbreeding did not affect offspring performance in either *S. pratensis* or *H. radicata*, confirming the results of other studies of the same species, which found that selfing mainly reduced the number of seeds produced and that late life-history traits were not affected by inbreeding depression (Pico et al., 2004 on *H. radicata*, Vergeer, Sonderren, & Ouborg, 2004 on *S. pratensis*).

Low soil pH had a very clear negative effect on the performance of *S. pratensis* and *H. radicata* offspring. Given that fragmentation often co-occurs with habitat deterioration in the Netherlands, due to eutrophication, acidification and desiccation (Vergeer et al., 2003), the performance of *S. pratensis* and *H. radicata* populations in habitats changing towards more acidic conditions

would drop rapidly. However, most of the performance traits of both species did not exhibit a significant pollination treatment \times soil pH interaction, indicating that inbreeding in these species does not increase their sensitivity to stress. Although other studies have suggested that effects of inbreeding depression in plant species are more pronounced under stressful conditions (Cheptou, Berger, Blanchard, Collin, & Escarre, 2000; Dudash, 1990; Hauser & Loeschke, 1996), our results clearly show that inbreeding by itself does not reduce offspring performance under acidic soil conditions.

Conclusions

Overall, we conclude that offspring performance traits in *S. pratensis* and *H. radicata* are not seriously affected by inbreeding. However, this study has demonstrated that the dispersal potential of *H. radicata* may be reduced by selfing and genetic drift, indicating that dispersal is not independent from the genetic erosion process. Variation among seed families and its interactions with most of the traits may indicate the populations' potential to adapt to new conditions. Furthermore, the performance of both species sharply decreased as soil conditions became more acidic, suggesting that both may decline in number when acidification of their natural habitat continues. However, inbreeding did not aggravate this process.

Acknowledgments

We are grateful to H. de Jong, R. Rengelink, A. Borghuis, F. Knauer, E. Jongejans, J. Roelofs, L. van der Berg, and M. Versteeg for greenhouse assistance, technical advice and various comments and suggestions during the progress of this work. We would also like to thank Het Limburgs Landschap for granting us permission to work in the De Hamert nature reserve. We are also grateful to Nick Waser, Johannes Kollmann and an anonymous referee for very helpful comments to improve the manuscript. This study was supported by the Netherlands Organisation for Scientific Research (NWO project 805-33-451) and by the TRANSPLANT (EVK2-1999-00042) EU project.

References

- Brown, J. H., & Kodric-Brown, A. (1977). Turnover rates in insular biogeography: Effect of immigration and extinction. *Ecology*, *58*, 445–449.
- Byers, D. L., & Waller, D. M. (1999). Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics*, *30*, 479–513.
- Cheptou, P.-O., Berger, A., Blanchard, A., Collin, C., & Escarre, J. (2000). The effect of drought stress on inbreeding depression in four populations of the Mediterranean outcrossing plant *Crepis sancta* (Asteraceae). *Heredity*, *85*, 294–302.
- Charlesworth, B., & Charlesworth, D. (1999). The genetic basis of inbreeding. *Genetical Research Cambridge*, *74*, 329–340.
- Clay, K. (1982). Environmental and genetic determinants of cleistogamy in a natural population of the grass *Danthonia spicata*. *Evolution*, *36*, 734–741.
- Cody, M. L., & Overton, J. McC. (1996). Short-term evolution of reduced dispersal in island plant populations. *Journal of Ecology*, *84*, 53–61.
- Crawley, M. J. (1997). Life history and environment. In M. J. Crawley (Ed.), *Plant ecology* (pp. 72–132). Oxford, UK: Blackwell Science.
- Dudash, M. R. (1990). Relative fitness of selfed and outcrossed progeny in a self-incompatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): A comparison in three environments. *Evolution*, *44*, 1129–1139.
- Dudash, M. R., & Fenster, C. B. (2000). The role of breeding system and inbreeding depression in the maintenance of an outcrossing mating strategy in *Silene virginica* (Caryophyllaceae). *American Journal of Botany*, *88*, 1953–1959.
- Ellstrand, N., & Elam, D. R. (1993). Population genetic consequences of small population size: Implications for plant conservation. *Annual Review of Ecology and Systematics*, *24*, 217–242.
- Fryxell, P. A. (1957). Mode of reproduction of higher plants. *Botanical Review*, *23*, 135–233.
- Grime, J. P., Hodgson, J. G., & Hunt, R. (1988). *Comparative plant ecology: A functional approach to common British species*. London: Unwin Hyman.
- Hagerup, O. (1954). Thrips-pollination in *Hypochaeris radicata*. *Nytt Magasin for Botanikk*, *3*, 55–58.
- Hauser, T. P., & Loeschke, V. (1996). Drought stress and inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae). *Evolution*, *50*, 1119–1126.
- Hooftman, D. A. P., Van Kleunen, M., & Diemer, M. (2003). Effects of habitat fragmentation on the fitness of two common wetland species *Carex davalliana* and *Succisa pratensis*. *Oecologia*, *134*, 350–359.
- Hufford, K. M., & Mazer, S. J. (2003). Plant ecotypes: Genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution*, *18*, 147–155.
- Ingarvarsson, P. K. (2001). Restoration of genetic variation lost – The genetic rescue hypothesis. *Trends in Ecology and Evolution*, *16*, 62–63.
- Koelwijjn, H. P. (1998). Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. *Evolution*, *52*, 692–702.

- Lienert, J., & Fischer, M. (2004). Experimental inbreeding reduces seed production and germination independent of fragmentation of populations of *Swertia perennis*. *Basic and Applied Ecology*, *5*, 43–52.
- Luijten, S. H., Kéry, M., Oostermeijer, J. G. B., & den Nijs, J. C. M. (2002). Demographic consequences of inbreeding and outbreeding in *Arnica montana*: A field experiment. *Journal of Ecology*, *90*, 593–603.
- Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution*, *45*, 622–629.
- Media Cybernetics. (1999). *Optimas release 6.5 Media Cybernetics*. Maryland: Silver Spring.
- Mix, C., Picó, F. X., & Ouborg, N. J. (2003). A comparison of stereomicroscope and image analysis for quantifying fruit traits. *Seed Technology*, *25*, 12–19.
- Olivieri, I., Swann, M., & Goyon, P. H. (1983). Reproductive system and colonizing strategy of two species of *Carduus* (Compositae). *Oecologia*, *60*, 114–117.
- Olivieri, I., Michalakis, Y., & Gouyon, P. H. (1995). Metapopulation genetics and the evolution of dispersal. *American Naturalist*, *146*, 202–228.
- Picó, F. X., Ouborg, N. J., & van Groenendael, J. M. (2004). Influence of selfing and maternal effects on life-cycle traits and dispersal ability in the herb *Hypochaeris radicata* (Asteraceae). *Botanical Journal of the Linnean Society*, *146*, 163–170.
- Richards, C. M. (2000). Inbreeding depression and genetic rescue in a plant metapopulation. *The American Naturalist*, *155*, 383–394.
- Roach, D. A., & Wulff, R. (1987). Maternal effects in plants. *Annual Review of Ecology and Systematics*, *18*, 209–235.
- Shaw, R. G., & Waser, N. M. (1994). Quantitative genetic interpretations of postpollination reproductive traits in plants. *The American Naturalist*, *143*, 617–635.
- Schmitt, J., Ehrhardt, C. W., & Schwartz, D. (1985). Differential dispersal of self-fertilized and outcrossed progeny in jewelweed (*Impatiens capensis*). *The American Naturalist*, *126*, 570–575.
- Schultz, S. T., & Willis, J. H. (1995). Individual variation in inbreeding depression – The roles of inbreeding history and mutation. *Genetics*, *141*, 1209–1223.
- Soons, M. B. (2003). *Habitat fragmentation and connectivity: Spatial and temporal characteristics of the colonization process in plants*. Ph.D. thesis. Utrecht: Utrecht University.
- Soons, M. B., & Heil, G. W. (2002). Reduced colonization capacity in fragmented populations of wind-dispersed grassland forbs. *Journal of Ecology*, *90*, 1033–1043.
- Soons, M. B., Heil, G. W., Nathan, R., & Katul, G. G. (2004). Determinants of long-distance seed dispersal by wind in grasslands. *Ecology*, *85*, 3056–3068.
- Vergeer, P., Rengelink, R., Ouborg, N. J., & Roelofs, J. G. M. (2003). Effects of population size and genetic variation on the response of *Succisa pratensis* to eutrophication and acidification. *Journal of Ecology*, *91*, 600–609.
- Vergeer, P., Sonderren, E., & Ouborg, N. J. (2004). Introduction strategies put on the test: Local adaptation versus heterosis. *Conservation Biology*, *18*, 812–821.
- Waser, N. M., Price, M. V., & Shaw, R. G. (2000). Outbreeding depression varies among cohorts of *Ipomopsis aggregata* planted in nature. *Evolution*, *54*, 485–491.
- Young, A. G., & Clarke, G. M. (2000). *Genetics, demography and viability of fragmented populations*. Cambridge: Cambridge University Press.