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Regional gene flow and population structure of the wind-dispersed plant species *Hypochaeris radicata* (Asteraceae) in an agricultural landscape

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Abstract

Using microsatellites, we investigated population structure and gene flow of the short-lived, wind-dispersed plant species *Hypochaeris radicata* in a fragmented agricultural landscape where more than 99% of the nutrient-poor grasslands have disappeared over the last century. We sampled populations in the few remaining high density populations in conservation areas, as well as individuals that occurred, with lower densities, in linear landscape elements, at two spatial scales. In a re-inventory of the landscape, after 3 years, both extinctions and colonizations of populations were observed. Contrary to expectations, no differences in genetic diversity between high and low density populations were observed. Both types of populations had relatively high levels of diversity. Overall genetic differentiation (θ) was 0.04 and significantly different from zero ($P < 0.01$). A significant isolation-by-distance pattern was found when all populations were simultaneously analysed ($r = 0.24$, $P = 0.013$). Isolation by distance was (marginally) significant at the small scale ($r = 0.32$, $P = 0.06$), whereas nonsignificant at the large spatial scale ($r = -0.05$, $P = 0.66$). A maximization-of-explained-variance procedure resulted in a threshold distance of 3.5 km above which populations were effectively genetically isolated. An additional partial exclusion Bayesian-based assignment test showed that overall 32.3% of the individuals were assigned to their population of origin, 48% were assigned to another population in the area and 19.7% were not assigned. Together, these results suggest high levels of gene flow. Seed dispersal contributes to the observed gene flow up to several hundred metres, which is higher than previously modelled using aerodynamic models on seed dispersal of *H. radicata*. We discuss the consequences of these results for an evaluation of the probability of persistence of this species in the fragmented landscape.

Keywords: agricultural landscape, Bayesian assignment, dispersal, gene flow, *Hypochaeris radicata*, metapopulation, microsatellites

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Introduction

Habitat fragmentation is generally recognized as a major threat to plant population persistence. A progressive reduction of suitable habitats has led to smaller populations separated by often unbridgeable unsuitable habitats. As a result, populations face the consequences of demographic,

environmental and genetic stochasticity (Schaffer 1987), increasing the probability of extinction (Lande 1993; Schemske *et al.* 1994). Genetic stochasticity, for instance, can negatively affect population persistence. Random changes in allele frequencies can lead to a loss of alleles or to the fixation of deleterious mutations, resulting in reduced genetic diversity (Hartl & Clark 1989) and lower fitness of the offspring (Byers & Waller 1999). The result is genetic divergence between populations and an alteration of regional population structure and metapopulation dynamics (Hutchison & Templeton 1999).

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Gene flow can counterbalance the reduction in genetic diversity of individual populations by bringing in new genetic material through seeds and pollen (Young *et al.* 1996). Dispersal of genetic material through seeds enables the founding of new populations, while dispersal through pollen can bridge existing populations, often over greater distances. In a fragmented landscape, the success of these two processes depends on a number of factors. For example gene flow can be limited as a result of lower plant densities after habitat fragmentation (Griffin & Barrett 2004). Seed dispersal, moreover, depends very much on a species' dispersal characteristics. For example, wind-dispersed plumed seeds have a lower terminal velocity and travel greater distances than plumeless seeds (Soons & Heil 2002; Tackenberg 2003). Pollen dispersal greatly depends on pollen dispersal vectors. For example, plant–pollinator interactions for insect-pollinated species are often disrupted in fragmented habitats, leading to reduced pollen transfer by pollinators (Kwak *et al.* 1998; Lennartsson 2002). The effectiveness of pollinators may also be affected by plant densities as indicated by a lower number of pollinators in populations with a lower density (Karron *et al.* 1995; Kunin 1997), which may result in lower outcrossing rates (Van Treuren *et al.* 1994). Pollinators are especially important for gene flow in species that are self-incompatible (Goodell *et al.* 1997).

Thus, while successful gene flow could rescue populations from genetic erosion (Brown & Kodric-Brown 1977), isolation between habitat patches aggravates the problem, not only because it reduces gene flow but also because it changes dispersal ability in an evolutionary sense through selection against dispersal in patches isolated in a hostile environment (Cody & Overton 1996). Given the importance of gene flow for the future persistence of plant species, there is a particular need for studies to provide measures of dispersal (i.e. gene flow) at various spatial scales.

Since direct estimates of realistic dispersal rates are difficult to obtain, it has been more common to apply indirect methods such as the use of molecular markers to estimate gene flow (Ouborg *et al.* 1999). Traditionally, F_{ST} values (or related measures of population differentiation) have been widely used to infer the number of migrants (Nm) and so determine dispersal. The reliability of such population differentiation measures, however, has been questioned (Whitlock & McCauley 1999), because these measures rely on unrealistic assumptions (Cain *et al.* 2000 and references therein). More recent studies have demonstrated that assignment tests or assignment-based differentiation measures are more appropriate in determining particularly contemporary levels of gene flow (Tero *et al.* 2003; Berry *et al.* 2004; He *et al.* 2004; Manel *et al.* 2005).

In the present study, we used highly polymorphic microsatellite loci and assignment procedures to investigate

gene flow and population structure of *Hypochaeris radicata* (Asteraceae) at two different spatial scales within a highly fragmented agricultural landscape. Within the study area, and equally distributed over both spatial scales, two types of populations are present: high density populations occurring in conservation areas under strict management, and low density populations occurring in nonprotected road verges. The investigated landscape therefore provides an ideal situation to study population structure in relation to spatial scale and to compare levels of genetic diversity between high density and low density populations after habitat fragmentation.

Hypochaeris radicata is a short-lived, mainly outcrossing and insect-pollinated herb that does not form a seed bank (Fryxell 1957; Aarssen 1981). The species produces single-seeded fruits equipped with a plumose pappus that is attached to a beak, allowing dispersal by wind (Soons *et al.* 2004). Seed dispersal distance calculations based on aerodynamic properties of the seeds have shown that most of them would not disperse more than 100 m, whereas a small fraction may bridge distances up to 400 m (Soons *et al.* 2004). Previous studies have shown that *H. radicata* is sensitive to inbreeding on seed and dispersal traits (Picó *et al.* 2004; Mix *et al.* 2006) and that the species' colonization capacity is reduced in fragmented landscapes (Cody & Overton 1996; Soons & Heil 2002). These characteristics indicate that the species may be sensitive to habitat fragmentation. This can have two effects: low density populations may show reduced genetic diversity, and increased interpopulation distances may reduce the level of gene flow. Thus, we expect the low density populations to have higher inbreeding levels and increased genetic differentiation with other populations. We also expect that population differentiation may be more evident at the larger spatial scale.

Materials and methods

Study site

The Achterhoek is a region of about 40 by 30 km², located in the mid-eastern part of the Netherlands. About a century ago, the vegetation consisted predominantly of nutrient-poor and moist, sandy, species-rich grassland (Nardo-Galion community). Intensive farming has reduced the former nutrient-poor vegetation by as much as 99.9% (Soons *et al.* 2004), and species-rich sites nowadays occur as small, isolated fragments, managed as conservation areas. *Hypochaeris radicata* L. still occurs in this region as an abundant species present in conservation areas in high densities (> 5 individuals/m²), as well as in linear elements like roadside ditches, the banks of waterways and railway tracks where the species has a low density (< 0.02 individuals/m²).

Fig. 1 Map of the Achterhoek region, showing the *Hypochaeris radicata* populations of the large- and small-scale sampling schemes in 2001. The dynamics of these populations has been visualized by also showing the distribution of populations in the small-scale sampling area in 2004.

Sampling strategy

In 2001, we sampled populations at two different scales (Fig. 1). First, we sampled all populations ($n = 9$) in an area of 2 by 2 km (small scale), chosen based on the maximum dispersal distance reported for *H. radicata* (Soons *et al.* 2004). Second, we additionally sampled a random selection of eight populations within an area of approximately 11 by 10 km (large scale). We defined a population as a group of individuals of *H. radicata* separated from the neighbouring group by a distance of at least 400 m. All populations have small population sizes (below 150), except for the population HKM (50 000) and HKD (600). Variation between population sizes was insufficient for further analysis.

The geographical distances between the populations in the small-scale study varied from 0.5 to 3.35 km. All individuals in the HKM1, HKM3 and HKM5 populations were sampled. Since the HKM2 and HKM4 populations

had relatively high plant densities, we sampled 33 and 37 individuals in these populations every 2 m along a linear transect. In order to determine the spatial dynamics [cf. extinction and (re)colonization] of populations, we revisited the small-scale sampling area in 2004 and screened all possible sites and linear elements again for the presence or absence of *H. radicata* (Fig. 1).

Within the large-scale study, the distances between sampled populations varied from 0.45 km to 11.0 km; note that four populations are part of both the small-scale and the large-scale analysis (Table 1). Here we sampled between 12 and 30 individuals in each population, again by taking a sample every 2 m along a linear transect, except for populations HE, HKE, HN, and HMA where all individuals were sampled.

At both scales of study both high density ($n = 3$ for small scale and $n = 4$ for large scale) and low density populations were sampled and analysed (Table 1).

Table 1 Characterization of the *Hypochaeris radicata* populations in the Achterhoek region sampled in the large-scale scheme (LS: 12 populations over an area of 110 km²) and the small-scale scheme (SS: nine populations within 4 km²)

Population name	Code	Geographical coordinates	Plant density	No. of plants sampled	Sampling strategy		
					LS	or	SS
De Hare	HDH	51°59'22"N, 6°32'41"E	Low density	30	LS		
Konijnendijk greppel	HKG	52°02'38"N, 6°26'09"E	Low density	30	LS		
Eibergseweg	HE	52°04'49"N, 6°34'49"E	Low density	20	LS		
Kempersweg	HKE	52°02'31"N, 6°32'18"E	Low density	17	LS		
Kooierlaan	HKL	52°05'05"N, 6°31'80"E	Low density	21	LS		
Het Spoor	HS	52°04'06"N, 6°28'43"E	Low density	28	LS		
Konijnendijk	HKD	52°02'06"N, 6°26'53"E	High density	30	LS		
Sonderenweg	HSO	52°04'30"N, 6°34'00"E	Low density	30	LS		
Molenweg	HM	52°01'43"N, 6°34'53"E	Low density	30	LS		SS
Nijkampseheide	HN	52°01'51"N, 6°32'41"E	High density	23	LS		SS
Maatweg	HMA	52°00'45"N, 6°32'25"E	High density	12	LS		SS
Koolmansdijk	HKM	52°01'13"N, 6°33'13"E	High density	30	LS		SS
Around HKM 01–31	HKM1	52°01'16"N, 6°33'44"E	Low density	31			SS
Around HKM 32–64	HKM2	52°01'01"N, 6°33'14"E	Low density	33			SS
Around HKM 65–76	HKM3	52°01'27"N, 6°32'53"E	Low density	12			SS
Around HKM 77–113	HKM4	52°01'43"N, 6°33'03"E	Low density	37			SS
Around HKM 114–124	HKM5	52°01'39"N, 6°32'34"E	Low density	11			SS

DNA isolation and microsatellite application

In May 2002, leaf material of 11 up to 37 individuals from the 17 populations (Table 1) was collected and kept at –80 °C until further analysis. DNA was isolated using DNeasy Plant Mini Kit (QIAGEN). The plants were genotyped using five microsatellite loci (HrGA9, 10, 12, 14 and HrGT4; Mix *et al.* 2004). Analysis of the complete data set showed significant linkage between HrGA9 and HrGA12, as was also found previously in another data set (Mix *et al.* 2004). Preliminary analyses showed that excluding either HrGA9 or HrGA12 led to the same results, therefore we decided to exclude HrGA12 for which a few missing values were present in the data set. Amplifications were performed on a T3 Thermocycler (Biometra) following the protocols developed by Mix *et al.* (2004). Fragments were analysed on the LI-COR 4200 IR² DNA analyser (LI-COR). Bands were scored using the SAGA^{GT} software (LI-COR).

Effects of density on within-population diversity

The following measures of within-population genetic diversity were calculated for low density and high density populations: allele number A , allelic richness R_p , gene diversity (H_E) (according to Nei 1973), observed heterozygosity (H_O) and inbreeding coefficient F_{IS} per population and per locus using FSTAT version 2.9.3 (Goudet 1995, 2001). Allelic richness value R_t in the small-scale study was corrected for differences in sample size between populations by applying

the rarefaction procedure described by Kalinowski (2004) using the program HP-RARE (Kalinowski 2005). Heterozygote excess and deficit were computed per locus, per population and across all populations using GENEPOP 3.4 (Raymond & Rousset 1995). The program was also used to test for multilocus-based departures from Hardy–Weinberg, using 400 batches and 1000 iterations per batch. A sequential Bonferroni correction was applied (Rice 1989).

We tested for differences of genetic diversity measures between low and high density populations, using one-way ANOVAS. The mean values were normally distributed thus meeting ANOVA assumptions.

Effects of scale on between-population diversity

Separate statistical analyses were performed for the large-scale and small-scale studies. Note that four populations belong to both sets (Table 1) and therefore we did not test for differences between scale levels.

Using FSTAT, overall population differentiation (θ) per locus and per population were calculated according to Weir & Cockerham (1984). FSTAT provided the standard deviation of θ by jackknifing across loci (300 replicas were generated). A t -test was used to test for significant deviation of θ from zero.

Assignment tests are considered to be accurate tools to measure contemporary gene flow, being robust to assumptions about the mutation model underlying the molecular markers (Berry *et al.* 2004; Manel *et al.* 2005). Gene flow between pairs of populations was estimated using a partial exclusion Bayesian-based assignment test (Rannala &

Mountain 1997) implemented in the GENECLASS 2.0 software package (Baudouin *et al.* 2004). The default settings were applied and the simulations were based on 1000 simulated individuals. GENECLASS 2.0 produces the probability for each individual to belong to the reference population. The number of individuals assigned correctly was determined as the number of individuals with the highest probability assigned to the source population. Additionally, we determined the number of individuals assigned to neighbouring populations as well as those assigned to the rest of the source populations. One of the advantages of the partial exclusion Bayesian-based assignment test is that the true population of origin does not necessarily have to be part of the sampled populations (Manel *et al.* 2002), as is often the case for highly mobile species (Cegelski *et al.* 2003). This is likely to be relevant for *H. radicata* in the present study, as we did not sample all populations in the large-scale study. At the small scale, where all populations were sampled, we counted the number of individuals that were not assignable to any of the populations as a measure of dispersal from outside the small-scale area.

To correlate genetic distances with geographical distances, we first used the SPASSIGN program (Pálsson 2004) to calculate estimates of pairwise F_{ST} , termed 'pairwise population genetic distance (D_{LR})', based on the partial exclusion Bayesian method by Rannala & Mountain (1997). The program provided the exact test for pairwise population differentiation based on permutation tests. SPASSIGN includes a Mantel test for the association between D_{LR} and geographical distances. However, because this program uses geographical latitude and longitude coordinates only as degrees and minutes, it does not allow geographical distances to be analysed with the precision needed for the region we studied. Therefore, we used the Mantel test (Manly 1985) implemented in FSTAT to assess the correlation (in terms of Pearson correlation coefficient) between the D_{LR} -matrix as computed in SPASSIGN and the geographical distance per population pair matrix, as well as to test linear associations between the genetic and geographical distances between populations at both scales (10 000 permutations).

In a simultaneous analysis of both spatial scales we tested for variation in isolation-by-distance patterns between the two scale levels, by moving the threshold geographical distance that separates small-scale and large-scale categories, in such a way as to maximize the percentage explained variance (R^2) in the small-scale pattern.

Results

Population dynamics

The small-scale study area was sampled in 2001 and surveyed again in 2004. In 1999, the area adjoining HKM

was acquired by the Dutch National Forest Service (site A, Fig. 1). As part of a restoration project, this area was turf-cut in 2001, while in 2002 a cycle path and a ditch were constructed next to the HKM site (site B, Fig. 1), offering potential new habitat for *Hypochaeris radicata* in this small-scale area.

Except for HKM5, all populations sampled in 2001 had decreased in area, with decreases ranging from about 30% (HKM1) to 80% (HKM2). For HKM2, all individuals along one of the roads had disappeared and the number of plants along the ditch was reduced (Fig. 1). In 2004, we found four sites where *H. radicata* had newly established. At the restored sites close to HKM (A and B), the species had established the largest number of individuals (several hundreds). At two new sites (C and D), a few *H. radicata* plants had established in linear elements (Fig. 1).

Marker diversity

Large numbers of alleles (A) were detected for all microsatellite loci, varying from 18 at locus HrGA9 to 43 at locus HrGA10 (Table 2). Allelic richness after rarefaction

Table 2 Descriptive statistics for all loci (a) and all populations (b): measure of differentiation (θ), allele number (A), allelic richness after rarefaction (R_t), expected (H_E) and observed (H_O) heterozygosity and inbreeding coefficient (F_{IS}) per population, calculated following Nei (1973). An asterisk (*) indicates significant values at $P < 0.05$ after Bonferroni correction

	θ	A	R_t	H_E	H_O	F_{IS}
(a) Per locus						
HrGT4	0.027	28	3.34	0.88	0.80	0.089*
HrGA14	0.037	31	3.30	0.86	0.78	0.141*
HrGA10	0.070	43	3.42	0.88	0.82	0.045*
HrGA9	0.037	18	3.38	0.89	0.87	0.002*
(b) Per population						
HDH		15.8	3.55	0.92	0.81	0.12*
HKG		13.8	3.44	0.90	0.91	-0.01
HE		10.0	3.36	0.89	0.80	0.10*
HKE		7.5	3.05	0.81	0.84	-0.03*
HKL		11.8	3.30	0.87	0.81	0.07
HS		13.3	3.42	0.90	0.86	0.04*
HKD		14.3	3.47	0.90	0.86	0.05*
HSO		11.0	3.31	0.87	0.84	0.04
HM		13.0	3.38	0.89	0.87	0.01
HN		12.0	3.21	0.84	0.73	0.12*
HMA		7.5	3.00	0.80	0.70	0.12
HKM		15.3	3.46	0.91	0.78	0.14
HKM1		15.3	3.44	0.91	0.83	0.09*
HKM2		12.8	3.48	0.89	0.86	0.04
HKM3		10.8	3.42	0.90	0.79	0.12
HKM4		13.8	3.39	0.89	0.83	0.07
HKM5		6.3	3.40	0.92	0.84	0.09
Overall	0.040	12.0	3.36	0.88	0.82	0.04

Fig. 2 Results of the assignment test for *Hypochaeris radicata*. Bar diagram indicates the percentages of individuals per population correctly assigned to the source population (black bars), assigned to neighbouring populations (hatched bar), assigned to other sampled populations (dotted bar) and not assignable to any of the sampled populations (white bar). Since neighbouring populations were not sampled in the large-scale study, this category was excluded.

(R_t), computed across all loci, ranged from 3.30 (HrGA14) to 3.42 (HrGA10). Expected and observed heterozygosity (H_E and H_O) ranged from 0.86 (0.78) at locus HrGA14 to 0.89 (0.87) at locus HrGA19 (Table 2). θ values per locus ranged from 0.027 (HrGT4) to 0.07 (HrGA10). The inbreeding coefficient (F_{IS}) varied between 0.002 at locus HrGA9 and 0.141 at locus HrGA14 (Table 2).

Effects of density on within-population diversity

The differences between high density and low density populations were nonsignificant for all measures of genetic diversity with the exception of the observed heterozygosity. The mean number of alleles (A) $\pm$ standard error was 12.0 ± 0.69 per population (Table 2), 12.28 ± 1.74 in high density and 11.94 ± 0.78 in low density populations ($F_{1,15} = 0.04$, $P = 0.84$). Allelic richness after rarefaction (R_t) computed across all populations was on average 3.36 ± 0.04 , 3.29 ± 0.11 in high density and 3.38 ± 0.03 in low density populations ($F_{1,15} = 1.31$, $P = 0.27$). Within-population gene diversity (H_E) was on average 0.88 ± 0.01 , 0.86 ± 0.03 and 0.89 ± 0.01 in all, high density and low density populations, respectively ($F_{1,15} = 1.84$, $P = 0.19$). The inbreeding coefficient F_{IS} within populations across all loci was on average 0.07 ± 0.01 , and varied from 0.11 ± 0.01 in high density populations to 0.06 ± 0.01 in low density populations ($F_{1,15} = 0.35$, $P = 0.08$). Seven populations showed significant F_{IS} values ($P < 0.001$, Table 2), indicating significant departures from Hardy–Weinberg equilibrium. One population (HKE) showed heterozygote excess, whereas the other six showed some heterozygote deficiency (Table 2).

Average observed heterozygosity (H_O) was 0.82 ± 0.01 (Table 2) and was the only measure that differed significantly between high and low density populations (0.77 ± 0.04 and 0.84 ± 0.01 , respectively; $F_{1,15} = 8.28$, $P = 0.01$). Contrary to our expectations therefore, low density

populations had higher observed heterozygosity than high density populations.

Effects of scale on between-population diversity

The measure of population differentiation across all loci was 0.04 (SD = 0.003) for both the small-scale and the large-scale study and deviated significantly from zero ($t = 231.21$, $P < 0.01$), indicating that population differentiation was small but significant at both scales of study.

The Bayesian-based assignment test assigned an average of 32.3% of all individuals (26.9% in the small scale and 36.4% in the large scale) to their own source population (varying between 0.0% for HKM5 and 61.3% for HM). In all these cases the next most likely population of assignment was several orders of magnitude less likely, underlining the robustness of this percentage. An average of 19.7% of the individuals could not be assigned to any of the sampled populations (16.8% and 21.1% for large and small scale, respectively; Fig. 2). The assignment values of correctly assigned and not assignable did not differ significantly between the two scales ($F_{1,15} = 0.41$ and 0.52 ; $P = 0.53$ and 0.48 , respectively).

In the small-scale study, a 27.6% proportion was assigned to their nearest neighbour population (Fig. 2). Since the true neighbouring populations were not known for the large-scale study, we could not determine this percentage for that scale. The remaining individuals were assigned to one of the sampled (but not the neighbouring) populations, indicating gene flow between these populations. The large-scale populations had a significant higher (45.9%) proportion of individuals assigned to one of the rest populations than the populations at small scale (22.2%, $F_{1,15} = 14.35$, $P = 0.001$).

Genetic distances (D_{LR}) between populations differed widely, from 0.13 (between HKM3 and HKM4) to 13.61

Fig. 3 Correlation of pairwise genetic distances with geographical distances of all populations of *Hypochaeris radicata*. Genetic distances (D_{LR} computed in SPASSIGN) are based on Bayesian assignment values; geographical distances are the shortest distances between the populations (log km). Correlation coefficients were calculated separately for distances under and over 3.5 km. Correlation coefficients: under 3.5 km: $r = 0.45$, $R^2 = 0.19$, $P < 0.001$; over 3.5 km: $r = -0.25$, $R^2 = 0.06$, $P = 0.48$. Not shown are all samples across all distances: $r = 0.24$, $R^2 = 0.05$, $P = 0.013$ and small-scale samples only: $r = 0.32$, $R^2 = 0.10$, $P = 0.06$.

(between HMA and HSO). The exact test showed that the genetic differentiation of some of the population pairs was significant. According to the Mantel test, the correlation between the pairwise genetic distance and the geographical distance (log km), using the Bayesian genetic distance value D_{LR} , was not significant for the large-scale study (correlation coefficient $r = -0.05$, $R^2 = 0.03$, $P = 0.66$), but marginally significant for the small-scale study ($r = 0.32$, $R^2 = 0.10$, $P = 0.06$), and significant across all samples ($r = 0.24$, $R^2 = 0.05$, $P = 0.013$). This was consistent with the pattern found when using genetic distances determined as F_{ST} (not shown).

When recalculating isolation by distance for small- and large-scale patterns, based on the entire sample, the explained variance of the small-scale pattern was maximal at a threshold distance of 3.5 km ($r = 0.45$, $R^2 = 0.19$, $P < 0.001$ below 3.5 km and $r = -0.25$, $R^2 = 0.06$, $P = 0.48$ above 3.5 km, Fig. 3). These results indicate that population differentiation follows an isolation-by-distance pattern up to 3.5 km, with 19% of the genetic distance explained by geographical distance. Above 3.5 km, the genetic distance does not increase further with geographical distance (Fig. 3).

Discussion

Habitat fragmentation can alter patterns of gene flow and population structure (Young *et al.* 1996). Such changes may be site- and species-specific (Jacquemyn *et al.* 2003) and may depend on plant density (Van Treuren *et al.* 1994), while these effects may also differ when considering different landscape scales (Hutchison & Templeton 1999). *Hypochaeris radicata* is a common species in the highly fragmented and agricultural landscape we studied, and

its seeds can disperse over distances of several hundred metres (Soons *et al.* 2004). When revisiting the small-scale area after 3 years, considerable dynamics of populations was observed, as was expected in this short-lived species. Several newly established sites were found, and as the species does not form a seed bank (Aarssen 1981) this indicates that seeds are dispersing through the landscape.

Our main objective was to investigate the possible effects of habitat fragmentation on genetic structure within and between populations of this species. We hypothesized that part of these effects may be mediated via effects of plant density within populations. Populations with reduced plant density are expected to show reduced levels of genetic diversity due to lower outcrossing rates (Van Treuren *et al.* 1994) and limited gene flow (Griffin & Barrett 2004), both resulting from lower pollinator attractiveness of low density populations (Karron *et al.* 1995). However, in contrast to our expectations, we found no difference in genetic diversity between high and low density populations. F_{IS} values varied between -0.03 (HKE) and 0.14 (HKM), but were not related to plant density. Such differences in F_{IS} values then might be due to spatial Wahlund effects (Murren 2003 and references therein) as within-population spatial genetic structuring can be high in small populations (Ingvarsson & Giles 1999).

In all populations high levels of genetic diversity were observed. Similar results have been found in other studies. For example, Hogbin *et al.* (1998) and Van Rossum *et al.* (2004) measured the genetic diversity in habitat fragments of the outcrossing species *Grevillea barklyana*, *Primula veris* and *Primula vulgaris*. They found that both verge and nonverge populations, a classification mimicking our high density–low density classification, showed similar high levels of genetic diversity. Godt *et al.* (1997) suggested that high levels of genetic diversity are common for outcrossing, short-lived species, when levels of between-population gene flow are high. The absence of density effects and the throughout high levels of within-population diversity that we observed in this area may therefore indicate that populations are not isolated here.

This conclusion is corroborated by the between-population studies at the two spatial scales. The weak population differentiation we observed indicates that there were still substantial levels of gene flow. Up to 3.5 km, the gene flow pattern was consistent with isolation by distance, indicating that gene flow was limited by geographical distance and followed a stepping-stone model (Hutchison & Templeton 1999). Gene flow is expected to take place through both seed and pollen dispersal. Successful seed dispersal must include establishment at new sites, so there must be suitable colonization sites available within a fragmented landscape (Soons & Heil 2002). The observation of new populations being established in the region we studied between 2001 and 2004 indicates that successful seed

dispersal did take place at this scale, and was most probably responsible for parts of the isolation-by-distance pattern.

In an earlier study, Soons *et al.* (2004) modelled seed dispersal and calculated that the majority of the seeds would not disperse beyond 100 m, and only a very small fraction would disperse up to 400 m. In our study, the average distance between the populations within the small-scale area was about 1 km, with the shortest distance between neighbouring populations being 500 m in the small-scale study. Our results indicate that dispersal, either by pollen or by seeds, occurs over at least a distance of 500 m. This is supported by the results of the assignment test. Of the individuals in the small-scale study, 21.1% could not be assigned to any population, suggesting they immigrated from outside the study area. Moreover, an additional 22.1% of the individuals were assigned to non-neighbouring populations. While both pollen and seed dispersal could contribute to these results, the observation of newly established populations at a distance of more than 400 m strongly suggests that seed dispersal contributed at least partly to the assignment results. This would then indicate that seed dispersal occurs over greater distances than was estimated using aerodynamic models (Soons *et al.* 2004).

The discrepancy between the modelled dispersal distance and the indirectly measured dispersal distance might be due to the uncertainty in modelling the tail of the dispersal curve (Bullock & Clark 2000; Cain *et al.* 2000). Another important parameter in the seed dispersal model of Soons *et al.* (2004) is the seed release height, as seeds released above the level of the surrounding vegetation can travel longer distances. The majority of the *H. radicata* individuals occurred in linear landscape elements that were open and frequently mown. The seed release height in such habitats may be much higher than in the dense grassland populations that were used to model seed dispersal distances for *H. radicata* (Soons & Heil 2002; Soons *et al.* 2004).

We made an effort to further analyse the overall relationship between geographical and genetic distance. While the overall relationship was significant, our detailed analysis showed that beyond 3.5 km, population differentiation did not follow an isolation-by-distance pattern (Fig. 3). Thus, gene flow exerts its influence up to 3.5 km; above that distance, genetic drift within populations has a larger influence than dispersal between populations (Hutchison & Templeton 1999). Populations farther apart than 3.5 km are therefore effectively, genetically isolated from one another.

The use by *H. radicata* of the linear landscape elements as habitats may positively affect gene flow. Recent studies have shown that insects tend to follow these elements rather than crossing monocultured, nonflowering farming areas (Kwak *et al.* 1998; Dover & Fry 2001; Tewksbury *et al.* 2002; Cranmer 2004; Townsend & Levey 2005). Thus the relatively high threshold distance of 3.5 km we found may

be specific for the present landscape and might be lower in other landscapes lacking the linear elements.

Consequences of habitat fragmentation for H. radicata

Hypochaeris radicata is characterized as being short-lived, has relatively high population turnover, seed dispersal distances up to several hundred metres, relatively high dispersal (gene flow) rates and no seed bank. These life history features all contribute to the possibility of metapopulation dynamics (Ehrlén & Eriksson 2003; Ouborg & Eriksson 2004). The species is present in discrete habitat patches and migration between these patches is common. From the life history and landscape descriptions, and the fact that the migration patterns did not fit any other model (as argued by Freckleton & Watkinson 2002 and Tero *et al.* 2003), we conclude that the species persists within the highly fragmented landscape as a true metapopulation (Ouborg & Eriksson 2004), using linear landscape elements as temporary habitats. The metapopulation dynamics is shown by the relatively large differences found in area covered and location, with local sites where the species had been decimated and other sites that had been colonized when the same region was surveyed 3 years later.

Modelling has suggested that the evolution of dispersal is favoured by succession in a metapopulation context (Olivieri *et al.* 1995) and as a result of changed ecological conditions during succession (Ronce *et al.* 2005). Our study shows that such regional dynamics occur for *H. radicata* in the Achterhoek region, which may confirm that the dispersal ability of this species might be subject to evolutionary changes. One of the best examples demonstrating short-term evolution of dispersal traits is that found in the study by Cody & Overton (1996), who found evidence for the hypothesis that dispersal traits (in *H. radicata*) might be liable to evolutionary change. However, they did not prove to what extent gene flow really occurs, nor did they prove whether the islands they studied were effectively isolated, or that dispersal has a genetic basis. Our results show that population isolation of *H. radicata* occurs from a distance of 3.5 km onwards. Results of an ecological study on *H. radicata* (unpublished results) strongly suggest that dispersal traits may have a genetic basis. Both findings confirm the possibility that Cody & Overton (1996) did indeed observe short-term evolution in *H. radicata*.

In the introduction we argued that *H. radicata* would be sensitive to habitat fragmentation because of its mating system (depending on pollinators), the fact that inbreeding reduces seed set and seed dispersal (Picó *et al.* 2004; Mix *et al.* 2006), the calculated maximum dispersal distances of 400 m (Soons *et al.* 2004), and the lower plant density of most of the populations. The genetic results, however, suggest that habitat fragmentation has led to a weak population differentiation, with populations maintaining their

levels of genetic diversity through extensive gene flow by pollen and seeds. Seeds of the species also seem to disperse over considerably greater distances than the 400 m suggested previously. Therefore, we feel justified in claiming that *H. radicata* shows an isolation-by-distance pattern and successfully survives habitat fragmentation as a metapopulation by using suitable but transitory habitat fragments such as linear landscape elements.

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Supplementary material

The supplementary material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/MEC/EC2887/MEC2887sm.htm>

Table S1 Pairwise geographical distances (km, below diagonal) and genetic distances (D_{LR} , in bold, above diagonal) between populations of *Hypochaeris radicata* in the large-scale (upper panel) and small-scale (lower panel) sampling schemes. Most of the population pairs were not significantly differentiated; those that were significantly differentiated are indicated by an asterisk * ($P < 0.05$).

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This study is part of Carolin Mix's PhD thesis on the genetic effects of habitat fragmentation on plant populations. Jan van Groenendael is head of the Department of Ecology and interested in population dynamics and population genetics of plant species and the conservation of biodiversity in fragmented landscapes. Joop Ouborg, as an Assistant Professor, is interested in molecular ecology of plants and animals and eco-dynamics of life-history traits. Ramses Rengelink is a research assistant. René Smulders and Paul Arens are (senior) researchers and share a common interest in studies on genetic diversity, population genetics and conservation biology issues.