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Demographic and genetic invasion history of a 9-year-old roadside population of *Bunias orientalis* L. (Brassicaceae)

Abstract The population history of a 9-year-old roadside population of the invasive plant *Bunias orientalis* was reconstructed by demographic analysis including size, position, age (determined by herbchronology) and RAPD-PCR patterns of individual plants. We evaluated emerging patterns of population growth and genetic structure during a full period of population development under typical site conditions (anthropogenic disturbance) and their possible consequences for the invasion potential of the species. The population has grown rapidly and continuously (though with slowing geometric population increase) during the 9 years since its foundation, filling the space available in the study area. Genetic variation (RAPD markers) was already high in the founder cohorts and remained at the same level throughout population development (variance fluctuations < 15%). Both results may be related to the mowing management at the site which seems to promote population growth of *B. orientalis* relative to other co-occuring species and to prevent the genetic drift and the development of spatial genetic structure that would be expected under isolation-by-distance models. Large founder plants had comparatively low genetic variance and were more closely related to younger cohorts than were small founder plants, indicating that selection acted during population development. Overall, the current anthropogenic disturbance regimes may contribute to high genetic variability by artificially increasing gene flow and thereby promoting the adaptability of invasive species to the often unpredictable conditions at disturbed sites. Our approach using retrospective demographic investigation allows the detection of spatio-temporal microscale patterns in genetic and phenotypic variation. Thus it allows a thorough understanding of local invasions of perennial herbaceous plants.

Key words Colonizing population · Disturbance effects · Herb chronology · RAPD variation · Spatial pattern

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Introduction

Invading species provide ideal "natural experiments" to study factors and processes that lead to population establishment and persistence (Harper 1977; Mack 1996). At the same time, understanding biological invasions may help to assess environmental risks posed by human-caused land-use change or genetic alterations of organisms (Drake et al. 1989; Crawley et al. 1993; Williamson 1996). Insight into processes underlying the successful establishment of invaders may also further our understanding of why many species are declining, and may therefore contribute to more effective conservation management of endangered species (Schmid and Matthies 1994).

Plant invasions can be described by a sequence of distinguishable phases: the arrival of propagules in a new area, establishment of populations (usually coinciding with a lag phase of slow increase), and beginning and sustained rapid expansion of both sizes and numbers of populations (e.g. Bazzaz 1986; Jäger 1988; Weber and Schmid 1993, 1998; Hobbs and Humphries 1995; Wade 1997). In addition to dispersal, a key process for plant invasions is successful establishment at new sites. Ideally the first years of population development lead to frequency distributions in age, number and size of individuals that make the population resilient and stable. Thus, investigations of patterns of initial demographic development, as determined by the interactions of plant

life-history traits with important site factors such as disturbance intensity, may be a key approach to understanding plant invasiveness.

In the absence of direct observations it is desirable to reconstruct the history of this demographic development from the current population structure (Dietz and Ullmann 1998; Schläpfer and Fischer 1998). For example, tree rings allow the ageing of trees in temperate zones (dendrochronology, Schweingruber 1996) and reconstruction of the dynamics of tree populations (Stoll et al. 1994). Recently it was suggested that age determination is also possible for many dicotyledoneous herbaceous plants of the temperate zone by counting annual growth rings in roots (Dietz and Ullmann 1997a). This so-called herbchronology facilitates investigations of local invasion processes of perennial herbs by simple surveys and helps to avoid time-consuming monitoring of marked cohorts (see Dietz and Ullmann 1998).

In addition to specific life-history traits, phenotypic and genetic adaptability to new environmental conditions often seem to be involved in plant invasions (Baker and Stebbins 1965; Martins and Jain 1979; Crawley 1986), e.g. during establishment of a population in a new or heterogeneous environment (Fischer and Schmid 1998). In this respect inclusion of genetic analyses (e.g. DNA fingerprinting methods) in demographic investigations can help to understand the change in genetic variation during population establishment and growth and may be particularly useful for the reconstruction of demographic processes, e.g. by the comparison of patterns of spatial distribution of plants and of their relatedness.

Bunias orientalis L. (Brassicaceae), a perennial herb currently invasive in Central Europe, offers an ideal opportunity to reconstruct the invasion process at a local scale within a population, because the large and separate individuals of this species can be easily identified and their age can be determined with herbchronology (Dietz and Ullmann 1997a). *B. orientalis* presumably originates from south-west Russia (Tutin et al. 1993) and has recently been expanding in the area of Würzburg in Germany, where it is spreading in disturbed habitats, along roadsides and at the edges of arable land and vineyards.

For an invading roadside population of *B. orientalis*, we determined the ages (using herbchronology), spatial positions and sizes of 371 individuals and molecular genetic variation in a subsample of 131 individuals using randomly amplified polymorphic DNA (RAPD) banding patterns (see e.g. Steinger et al. 1996). In this way we could reconstruct the time course of changes in age structure, spatial structure and genetic structure. We asked the following questions:

1. Which pattern of spatial and numerical population development since the foundation of the population is revealed by herbchronology?
2. How do these patterns relate to the site factors?

3. Is there a correlation between spatial and genetic distance among individuals?
4. Is genetic variation related to size variation, i.e. are young individuals on average more closely related to large old individuals (presumed parents) than to small old individuals?

We also evaluated a hypothesized network of causal and correlational relationships among age, genetic and spatial variation, and size using structural equation modelling. Our main aims were (1) to obtain a more profound understanding of possible factors determining the invasiveness of *B. orientalis* and (2) to simultaneously explore the potential of our retrospective methodological approach to gain insights into the mechanisms of local invasions of perennial herbaceous plants.

Materials and methods

Species

B. orientalis is a semi-rosette, polycarpic, perennial hemicryptophyte. It is very plastic in size with adult rosette diameters of 10–100 cm. The species develops a permanent, fleshy tap root which produces vegetative offspring from dormant buds only if it is damaged or severed (Steinlein et al. 1996; Dietz and Steinlein 1998). Under typical conditions, i.e. in occasionally disturbed, nutrient-rich habitats, individual plants can reach the generative stage in their 2nd year and most of the generative plants remain in this stage for years, showing very low mortality (Dietz and Steinlein 1998). The plants can attain ages of more than 12 years (H. Dietz, unpublished work). In spring reproductive individuals develop flower stalks of 0.5–1.5 m height. Although it is known that the species shows mixed mating (Oberdorfer 1990), data on the amount of selfing are still lacking. Individual plants produce up to several thousand fruits (oval pods containing one or two seeds) and fruit fall can extend from late summer to spring of the next year. The species forms a persistent seed bank (Dietz and Steinlein 1998) but seeds can also germinate without a dormancy period following dispersal in autumn.

Study site

The study site is a roadside verge located in the Middle Main valley 7 km northwest of Würzburg (Unterfranken, Germany, 49°51′N, 9°51′E). The area is situated between the road and a hedgerow running almost parallel to the road at a distance of 8–11 m. It is characterized by herbaceous vegetation dominated by the grass *Arrhenatherum elatius* (L.) P. B. ex J. et C. Presl and the forbs *B. orientalis*, *Galium album* Mill. and *Picris hieracioides* L. The population of *B. orientalis* has developed mainly in a 5–7 m wide and 50 m long strip adjacent to the hedgerow. The site has been mown irregularly (on average once a year) during the whole period of population development of *B. orientalis*. Field observations indicated that the removal of litter after mowing always occurred parallel to the hedgerow.

Plant positions

In November 1997 a contiguous sampling area of 5–6 m × 16 m was arbitrarily chosen within the dense strip of the *B. orientalis* population adjacent to the hedgerow (Fig. 1). The positions of all *B. orientalis* plants which were found within the area were deter-

Fig. 1 Position and age of all *Bunias orientalis* plants within the study area. Individuals selected for genetic analysis are represented by *shaded symbols*. *Filled symbols* denote large founder plants
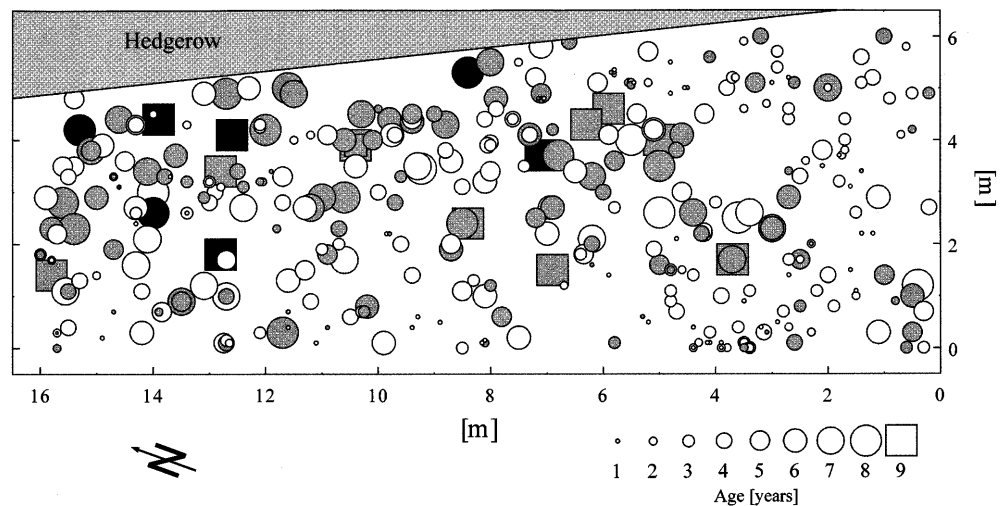
mined to the nearest 10 cm. The 384 established plants were harvested by severing the main roots 15–20 cm below the soil surface. Among the seedlings found (i.e. plants recruited in 1997) only a fraction may reach sufficient size to become established the next year (cf. Dietz and Steinlein 1998). They were, therefore, omitted from analysis.

Size and age

The size and age of the harvested plants were determined in the laboratory. In a regularly mown *B. orientalis* population in the Botanical Garden of the University of Würzburg the number of fruits produced per plant increased linearly with the number of shoots ($r^2 = 0.89$, $P < 0.001$, $n = 20$; H. Dietz, unpublished work). Therefore, as a fitness-related trait, the number of viable (well-developed) shoots per plant was noted. With one exception, 2- and 1-year-old plants had only one shoot. For these plants root diameter was measured at 10 cm depth.

Plant age was determined by counting annual growth rings in the cross-section of the root cut at 5–15 cm depth from the soil surface (herbchronology, Dietz and Ullmann 1997a). In 77% of all cases the age could be determined unambiguously, ranging from 1 to 9 years. Thirteen plants with central root decay were excluded from the analysis. The rest of the plants could be assigned to either one of two consecutive ages. Root fragments of the sampled plants were placed in plastic containers filled with loamy soil and were allowed to regenerate in the glasshouse for 7 weeks to obtain fresh material for the subsequent genetic analyses.

Classification of cohorts

Age structure analyses performed 2 years earlier in an adjacent section of the same population found that the oldest plants were 7 years old (Dietz and Ullmann 1998). Thus, the oldest individuals found in the present study (8- and 9-year-old plants) can be regarded as the founder plants of the population. The founding diaspores were probably introduced during road construction work adjacent to the study site in the period between 1985 and 1988. Individuals 1–7 years old are likely to be direct or indirect descendants of the founders or, less likely, may be immigrants from adjacent sections of the population or farther away.

RAPD analysis

The sampling area was subdivided into 15 equal-sized cells and in each cell one individual per annual age cohort was randomly se-

lected. If no individual of a given cohort was available in a particular cell, the required plant was drawn randomly from one of the remaining cells. For the two oldest age cohorts, 9- and 8-year-old plants, relatively few individuals were available. Thus, five individuals which could not be unambiguously assigned to either one of the two ages were included. The final set contained 26 plants that were 9- or 8 years old and 15 each of all younger age cohorts.

The genetic analyses were carried out at the Institut für Umweltwissenschaften, University of Zurich. Young leaves were rinsed with distilled water and freeze-dried (lyophilized) for 48 h. The dried leaves were placed in 2.0-ml Eppendorf tubes containing one glass bead and were ground to a fine powder in a shaking mill. DNA extraction followed a modified CTAB protocol (Steinger et al. 1996). PCR reactions (25 ml) contained PCR buffer solution [1×10^{-2} M Tris-HCl (pH 9.0), 5×10^{-2} M KCl, 0.08% nonidet P40], 2.5 mg BSA, 2.5×10^{-3} M MgCl$_2$, 4×10^{-4} M each of dATP, dCTP, dGTP and dTTP, 5×10^{-12} M RAPD primer (Operon Technologies Inc., Alameda, Calif., USA), 0.5 units Taq polymerase (MBI Fermentas, Lithuania) and approximately 20 ng of DNA (as determined with a Hoefer TKO 100 fluorometer, Hoefer Scientific Instruments, San Francisco, Calif., USA). The reaction solutions were pipetted into the wells of microtiter plates and covered with one drop of mineral oil. The solutions were incubated in a PCR thermocycler (PTC-100, MJ Research Inc., Watertown, Mass., USA) with the following program settings: 1 min at 93°C followed by 45 cycles of 30 s at 92°C, 30 s at 36°C and 90 s at 72°C. Incubation at 72°C (5 min) was included as a final step. DNA amplification products were electrophoresed on 1.4% agarose gels and stained with ethidium bromide. The gels were digitized on a UV table with a video camera-based imaging system (Inotech AG, Dottikon, Switzerland).

Twenty ten-base primers (Operon series B) were tested for reproducible banding patterns with four replicates of a single DNA extract. Eleven of these primers yielded reproducible fragment profiles and so were tested for the presence of polymorphisms using three DNA extracts obtained from five different plants. Seven primers (B07, B08, B11, B12, B15, B17 and B18), yielding polymorphic fragment patterns, were finally screened in all 131 DNA samples. Of the 40 scorable bands in the range of intermediate molecular weight (~500–1900 bp) 20 were polymorphic. The presence or absence of bands were scored and the results were coded as 1s or 0s yielding a binary data matrix with 131 rows and 20 columns.

Data analysis

AMOVA (analysis of molecular variance, Excoffier et al. 1992) was used to analyse variation among RAPD phenotypes. Based on squared Euclidean distances between RAPD phenotypes, AMOVA

calculates sums of squares and estimates variance components similarly to conventional ANOVA (as applied in Huff et al. 1993; Buso et al. 1998). AMOVA allows estimation of the relatedness among cohorts and tests their pairwise genetic distances (Φ_{st}) with a permutational approach (Excoffier et al. 1992). To describe the relatedness of plants within cohorts and therefore within-cohort genetic variation we use the measure "sums of squares per cohort divided by number of plants per cohort minus 1" ("molecular variance"; Fischer and Matthies 1998b). The analyses were performed using the software program AMOVA 1.55 provided by Laurent Excoffier (University of Geneva).

STATISTICA for Windows 5.1 (Statsoft Inc., 1997, Tulsa, Okla., USA) was used for all other analyses with values of $P < 0.1$ regarded as marginally significant and values of $P < 0.05$ accepted as significant. Generally, for significance tests involving permutation procedures (AMOVA and Mantel test) 999 permutations were run if $P \ll 0.05$ or $P \gg 0.05$. Otherwise 4999 permutations were run to obtain more accurate P-values (Manly 1997).

The relationship between genetic distance and spatial distance among individual plants was analysed with the Mantel test (Mantel 1967; Fortin and Gurevitch 1993) with squared Euclidean distances between pairs of RAPD phenotypes as a measure of genetic distance.

We investigated the relationships between time, genetic and spatial distance from founder individuals as explanatory variables, and plant size as fitness measure. To this end we established a path diagram which we tested with the 105 7- to 1-year-old individuals, i.e. with the potential descendants of the founder plants. Because of their assumed high influence on later cohorts we chose the seven largest founders (i.e. founder plants with ≥8 shoots) a priori as a reference group for the calculation of spatial and genetic distance. As directional removal of litter (approximately in x-direction) could have influenced spatial relationships within the population, we considered the x- and y-distance from large founders separately (calculated as the absolute difference to the mean x- and y-position of the large founders). Thus the resulting path diagram comprised age, x-distance, y-distance, mean squared Euclidean distance (MSED) of RAPD phenotypes to the seven large founder plants, and plant size. The number of shoots per plant was used as a size score. For 2- and 1-year-old plants root diameter (varying between 0.15 cm and 1.0 cm) was used as a surrogate for shoot number because size differentiation on the basis of shoot number was not possible. Distances were square-root transformed and plant size scores were log-transformed for analysis. The correlation matrix of the transformed variables is shown in Table 1.

In our path diagram we hypothesized that age influenced plant size and also that position accounted for an increase of the colonized area during population development. We also included a direct effect of x-distance on y-distance as y_{max} increased with increasing x due to the oblique border of the study area to the hedgerow (Fig. 1). To account for effects of limited dispersal both position coordinates were assumed to affect genetic distance to large founders. Position coordinates were also assumed to influence plant size because the large founders were mainly situated at high x- and y-values where population density was rather high (Fig. 1). We also incorporated a possible effect of genetic distance to large founders on plant size to account for relatedness. Except for age (the root of the path diagram) latent exogeneous variables (Steiger 1995) were assigned to all variables to include the effects of unexplained factors.

We used structural equation modeling (SEM), a multivariate analysis technique similar to but more general and more robust than conventional path analysis (Steiger 1995; Pugesek and Grace 1998), to analyse the set of assumed relationships between age, genetic and spatial distance to large founders and size. Arrows in the path diagram thus calculated represent suggested causal effects between the variables. SEM yields a set of path coefficients (standardized partial regression coefficients, pc) between the dependent and the predictor variables in the path model. By using SEM it is possible to simultaneously test the significance of all path coefficients included in the model, and whether the overall causal model departs significantly from the data or not. Furthermore, SEM allows the calculation of indirect effects by tracing connected paths (Mitchell 1993). We used generalized least squares as discrepancy functions followed by maximum likelihood estimation in the SEPATH module of STATISTICA and inspected the χ^2 statistic for the goodness of fit between the estimated and the sample covariance structure.

Results

Population structure

The continuous age distribution of plants of *B. orientalis* ranging from 9- to 1-year-old individuals (Fig. 2A) indicated that seedlings had successfully recruited in every year since the foundation of the population. The number of individuals per cohort seems to have increased almost linearly from <15 9-year-old plants to >60 1-year-old plants. (We cannot give precise figures because unambiguous ageing was not possible for all individuals and because a few established individuals may have died despite the generally very low mortality of *B. orientalis* during the initial years of population development.) The net number of established plants within the sampling area was about 25 times higher in 1997 (~400 individuals) than in 1989 (Fig. 2B). The annual rates of population increase (λ) declined from about 2.5 to values close to 1 over the whole course of population development (Fig. 2B). This is consistent with a slowing of geometric population increase as in logistic models.

Individuals of *B. orientalis* were distributed over the whole study area (Fig. 1). The median number of shoots per plant increased monotonically with age ($r = 0.99$, $P < 0.001$, $n = 9$; Spearman's rank correlation) although there was high within-cohort variation in plant size (Fig. 3). Founder plants tended to occur closer to the hedgerow than did younger plants. Most individuals of the youngest cohorts grew at the margins of the population (Figs. 1, 4). Population density in the central strip (2 m < y < 4 m) parallel to the hedgerow was high with more than five established individuals per square meter. Furthermore, there was a greater number of older (larger-sized) plants in this section suggesting that the plants, particularly individuals of the younger cohorts, suffered more from intraspecific competition than in the adjacent sections. Correspondingly, individuals located in the most densely populated strip showed a trend for smaller size as compared with those growing in the adjacent zones (Fig. 4).

Table 1 Correlation matrix of the transformed variables used in the path model shown in Fig. 7 ($n = 105$ plants). Distances were square-root transformed and plant size scores were log-transformed

	Age	x-position	y-position	Genetic distance	Size
Age	1.00				
x-position	−0.00	1.00			
y-position	−0.19	0.31	1.00		
Genetic distance	0.06	−0.03	−0.21	1.00	
Size	0.79	0.22	−0.07	0.16	1.00
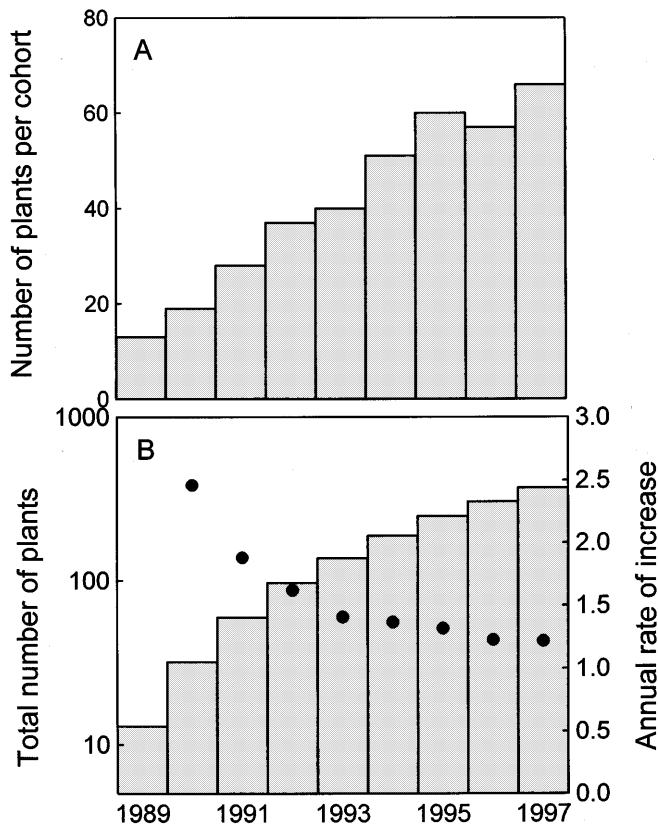
Fig. 2A,B Reconstruction of population development (*columns*) between 1989 and 1997 based on the age distribution of all 371 plants analysed. Plants which could only be assigned to either one of two consecutive ages were proportionally allocated to one of both ages. The annual rates of population increase (λ) are indicated by *filled circles*
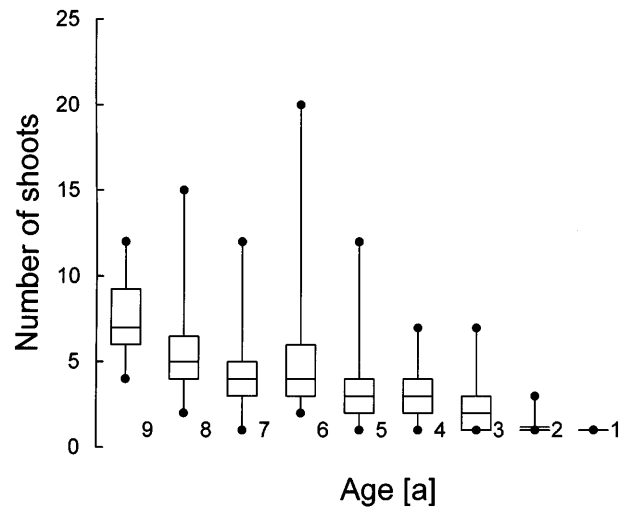

Fig. 3 Box-plot illustrating within-cohort range, quartiles, and median of plant size for all sampled plants

RAPD variation

The 131 plants belonged to 109 different RAPD-phenotypes. Differences between individuals within cohorts accounted for 89% of total molecular variance whereas only an additional 11% was due to differences between cohorts. Correspondingly, pairwise genetic distances (Φ_{st}) between cohorts were – at best – marginally significantly different from zero ($P > 0.06$), and overall differentiation among cohorts ($\Phi_{st} = -0.142$) was not significant ($P = 0.29$).

Molecular variance within cohorts did not change with cohort age (Fig. 5A) and molecular variance of the whole population did not change with the age of the population (Fig. 5B). This suggests that genetic variation was neither being depleted nor accumulating during the 9 years of population development.

Spatial pattern of genetic variation

Genetic distances and spatial distances among the 131 plants were not correlated (Mantel test, $r = 0.004$, $P > 0.5$). Semivariance analysis estimating spatial au-

tocorrelation for discrete spatial distance classes (e.g. Rossi et al. 1992; Robertson and Gross 1994) showed that there was no spatial autocorrelation of genetic distance. When we tested for differences in RAPD phenotypes between plants in the left half of the population ($x < 8$ m, $n = 69$) and plants in the right half ($x \geq 8$ m, $n = 62$) this explained only a non-significant 3.8% of total variance ($P = 0.69$). Genetic distance for all possible pairings of the plants of different cohort groups (founder plants; 7- and 6-year-old plants and 5- to 3-year-old plants) with their respective potential descendants (younger plants) was not significantly correlated with the corresponding spatial distance when grouped into intervals of 1 m ($|r| < 0.5$ m). These results indicate that genetic variation at the level of both the whole population and of cohort groups was independent of spatial variation.
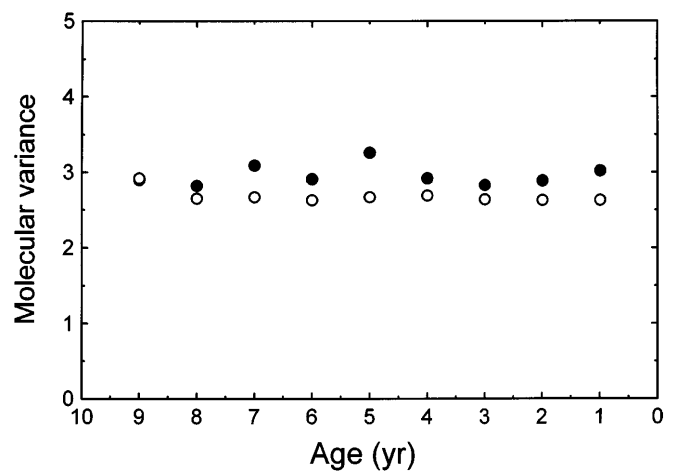
Correlation between size of founder plants
and genetic distance to their potential descendants

Mean genetic distance of plants of the founder cohorts to possible descendants was shorter (MSED 4.89 ± 0.04 SE) than mean genetic distance of 7- and 6-year-old plants or 5- to 3-year-old plants to their potential descendants (MSED 5.26 ± 0.04 SE or 5.23 ± 0.03 SE, respectively). There was a significantly negative correlation between the size of the founder plants and the genetic distance to all their possible descendants (6- to 1-year-old plants, Fig. 6; $P < 0.03$). This closer relatedness of younger cohorts to large founder plants indicates that large founder plants had high fitness and thus were the dominant ancestors of the population. Mean genetic distances to possible descendants were rather similar among large founders (Fig. 6; coefficient of variation 4.9%) whereas mean genetic distances to potential descendants varied considerably among the smaller foun-
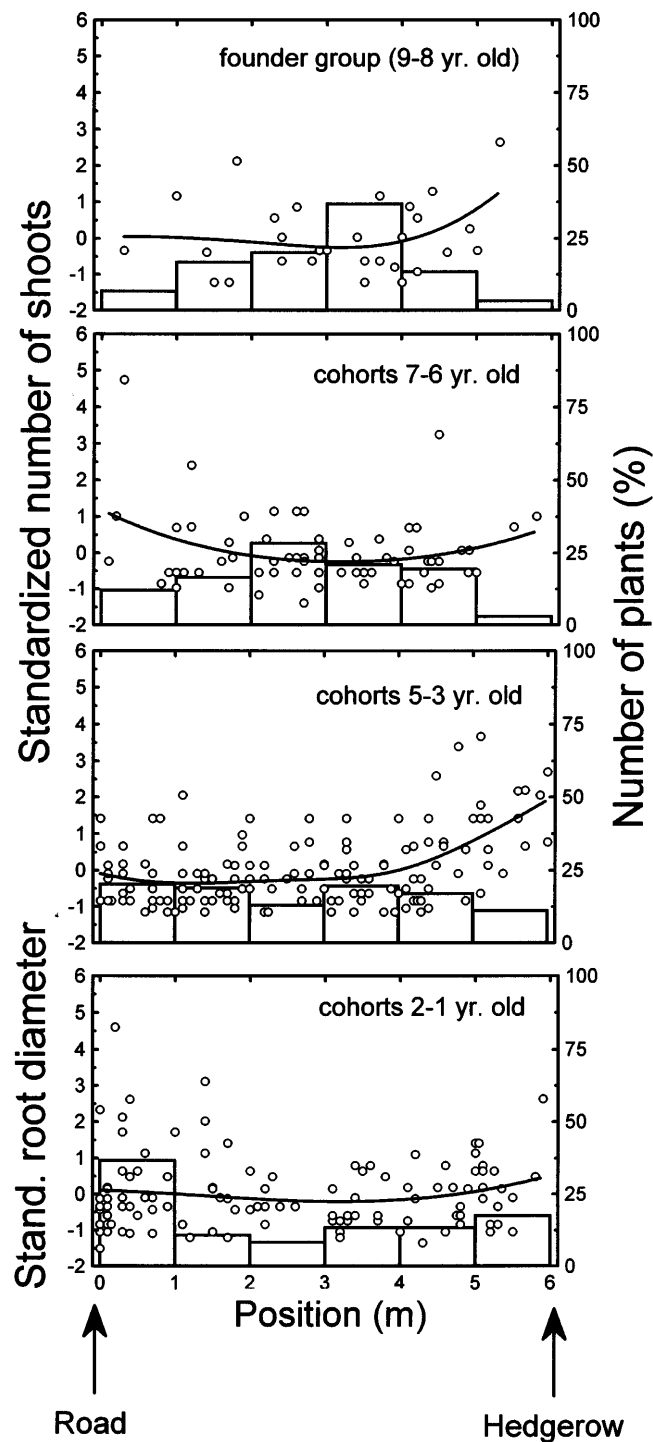
Fig. 4 Number and size of *B. orientalis* plants for four different cohort groups as a function of *y*-position (roughly perpendicular to the road and hedgerow, see Fig. 1). Size measures were standardized by substitution into $m = (y - y_{mean})/s$ where s is the standard deviation. Least square *regression lines* were fitted to the scatterplots (size of plants). The *histograms* indicate frequencies (number of plants) per 1-m step in *y*-direction

Fig. 5 Relationship between within-cohort molecular variance and cohort age (*filled circles*), and between whole-population molecular variance and population age (*open circles*). Molecular variance is the sum of squares of RAPD variation per cohort divided by $n-1$ (see methods)
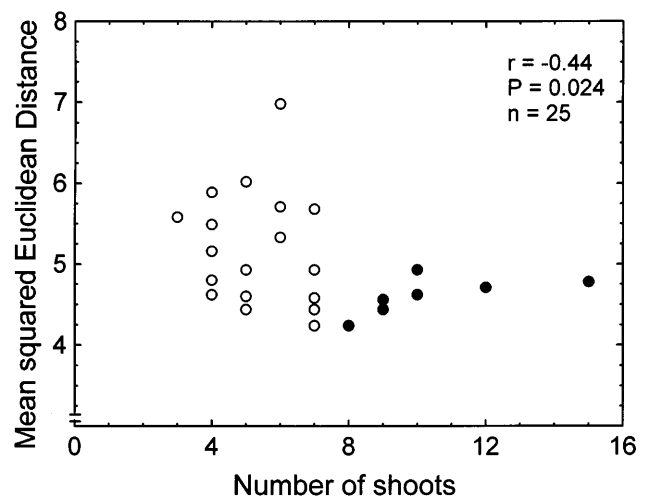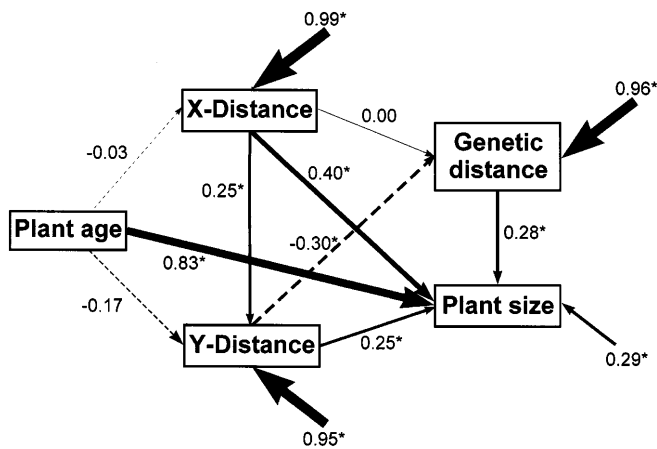

Fig. 6 Relationship between the size (number of shoots) of founder plants and their mean genetic distance (MSED) to all possible descendants (6- to 1-year-old individuals). Statistical parameters shown are obtained from Spearman's rank correlation test. *Filled circles* represent large founder plants (≥8 shoots)

$F = 10.1$, $df = 17$ (small founders), $df = 6$ (large founders), $P < 0.005$].

Relationship among the variables included in the path model

The path model showed a good overall fit to the data ($\chi^2 = 0.02$, $df = 1$, $P = 0.88$). Plant age strongly positively determined plant size (Fig. 7; standardized partial regression coefficient pc = 0.83; $P < 0.001$) and, consistent with Fig. 4, there was a non-significant trend for younger cohorts to be situated farther away in the *y*-direction from the mean position of large founders than were older cohorts (pc = −0.17; $P = 0.13$). Dis-

ders (coefficient of variation 13.3%). Accordingly, the corresponding variances were significantly different between the two groups [*F*-test for comparing variances;

Fig. 7 Path diagram of hypothesized relationships between plant age, spatial, and genetic distance to large founders, and plant size among 7- to 1-year-old individuals. *Arrow widths* correspond to the values of the path coefficients. *Arrows* that are not connected to a predictor variable indicate the effect of unexplained factors. *Dashed lines* show negative relationships. *Asterisks* denote path coefficients significantly different from 0

tance in x-direction was not related to plant age and had no effect on genetic distance between large founders and younger cohorts which is in accordance with the general independence between spatial variation and genetic variation. However, greater y-distance to large founders resulted in closer relatedness of younger plants to large founders (pc = −0.30; P < 0.002; see Discussion).

Plant size was greater the farther away the younger plants were located from the mean x-position of large founders (direct effect pc = 0.40; total effect 0.46; P < 0.001). Y-distance to large founders, however, had only a weakly positive effect on plant size because the significant direct effect of 0.25 was partly compensated for by an indirect negative effect via genetic distance of −0.09 (−0.30 × 0.28). Plant size was also positively related to genetic distance (pc = 0.28; P < 0.001), i.e. smaller plants among the younger cohorts were genetically more similar to large founders than were larger plants (see Discussion).

Overall, the path diagram indicated that plant size (plant fitness) of 7- to 1-year-old individuals was significantly related to all variables included in the model. Furthermore, directional spatial effects seem to have contributed to the development of genetic and size structure of the population.

Discussion

Population structure

The strong and continued population growth reconstructed in our study is in close agreement with the inferences drawn from demographic transition data from *B. orientalis* plants sampled at other disturbed habitats (Dietz and Steinlein 1998). Since its probable foundation

in 1988 the study population of *B. orientalis* has rapidly grown by yearly successful recruitment of seedlings which resulted in a broad age structure and in a continuously increasing occupation of the available area. The constant population increase has most likely been promoted by the moderate disturbance frequency of irregular mowing, advancing both seedling establishment and growth of adult plants (Dietz and Steinlein 1998; Dietz and Ullmann 1998). In a 1995 survey of an adjacent portion of the population of *B. orientalis* some of the 1-year-old plants, most of the 2-year-old plants, and all older (3- to 7-year-old plants) were reproductive (Dietz and Ullmann 1998). Furthermore, no dead rootstocks of *B. orientalis* were found in the study area of 1995 or in this study, indicating very low mortality of established plants. All these features fit the pattern expected of a viable, expanding population of an invasive plant species and they are in contrast to traits reported for populations of declining plant species with an excess of old plants (Oostermeijer et al. 1992, 1994), which grow slowly or have inefficient reproduction (Sih and Baltus 1987; Widén 1993; Fischer and Matthies 1998a), and which have high rates of mortality (Fischer 1996). Unless site management changes in the future, population density of *B. orientalis* most likely will further increase until density-dependent effects, shortage of essential resources, or disease stop population growth and might eventually cause degeneration. Longer-term persistence by both individual plant survival and annual seedling recruitment of the population of the perennial *B. orientalis* is in contrast to the dynamics of annual oilseed rape (*Brassica napus* ssp. *oleifera*) in roadside patches which are characterized by very quick turnover (Crawley and Brown 1995).

As a deeply rooting, semi-rosette species *B. orientalis* is a rather poor (above-ground) competitor when grown in mixtures with taller forbs (Dietz et al. 1998) but seems to suffer less from interspecific competition in grass-dominated vegetation. In this situation the species further profits from mowing because of its capacity for rapid and vigorous rosette regrowth (Dietz and Ullmann 1997b). As a result of mowing there were relatively many grass plants and few tall-growing forbs at the study site, suggesting that intraspecific competition was more important for the development of the size structure within the population than interspecific competition. Strong asymmetric intraspecific competition for light might have occurred between older plants with large rosettes that partly covered younger, smaller plants growing in the neighbourhood. This would explain why larger plants of younger cohorts tended to be found outside the domain of greater population density with a higher number of older plants (Fig. 4).

Genetic variation

Although only based on seven different primers, the RAPD-PCR banding patterns had a sufficiently high

resolution to discriminate different genotypes in the very small study area. Our analysis showed that all founder plants belonged to different RAPD-phenotypes, indicating that genetic variability within the population was already high at the beginning of the invasion at the study site. It further indicates that the founder plants represent recruits from seed rather than possible regenerates from fragmented root systems of one or a few plants.

RAPD markers are considered to be essentially selectively neutral (Bachmann 1994). Thus they are especially useful for detecting patterns of relatedness among individuals (with the caveat that some could be linked with genes under selection). Generally, estimates of population-genetic parameters based on RAPD have to be interpreted with caution because of their dominant nature (Lynch and Milligan 1994). Nevertheless, we consider our conclusions robust because the among-cohort genetic distances and differences in genetic variability were clearly not statistically significant. Due to their selective neutrality, RAPD markers are also appropriate for measuring the effects of genetic drift. On the other hand, RAPD variation may be a poor indicator of genetic variation in quantitative traits and therefore of the effects of selection (Storfer 1996).

After population foundation, subsequent change in genetic variability results from the interaction among selection, genetic drift, mutation and gene flow. Depending on the direction and relative importance of these forces genetic variability may decrease, remain constant, or increase. Directional selection in a homogeneous environment is expected to reduce genetic variation in fitness-related traits, whereas in a heterogeneous environment it may also result in constant or increased genetic variability, depending on the scale of gene flow and environmental heterogeneity (e.g. Mitchell-Olds 1992 and references therein). In our study there was evidence for selection because younger plants were more closely related to larger founders than to smaller founders. Genetic drift is expected to reduce genetic variability if the effective size of a population is small. In our study we found constant levels of variability both in RAPD variability (Fig. 5) and coefficients of variation in plant size (Figs. 3, 4) suggesting that both selectively neutral and quantitative variation were maintained in the study population of *B. orientalis* throughout the study period of 9 years. This maintenance of genetic diversity may have been mediated by a storage effect (Warner and Chesson 1985; Ellner and Hairston 1994) due to the long-lived, polycarpic life cycle and the persistent seed bank of *B. orientalis*, by the continuous growth of the population, or by gene flow through translocated seeds during litter removal after mowing of the road verge.

Fruit dispersal of *B. orientalis* is strictly local. Almost all fruits can be found within a radius of 2 m of the position of the mother plant (H. Dietz, personal observations), unless plant stands are situated on a steep slope or have a high amount of bare ground. In the related species *Brassica napus* pollen transfer is limited to distances <1 m in more than 95% of all cases (Scheffler et al. 1993). Thus, the development of a spatial genetic substructure by limited gene flow (isolation-by-distance), reported for many plant species (Slatkin 1987), could also be expected for *B. orientalis*. However, we found no general relation of genetic and spatial variation. The road verge including the study area is mown stripwise (strip width *c*. 1.5 m; T. Steinlein, personal communication), running parallel to the hedgerow. Thus, stripwise translocations of infrutescences and seeds prevalently in the *x*-direction may have obscured spatial genetic differentiation in *x*-direction. This is supported by the observation that position in the *x*-direction was not related to plant age. The rather obscure finding that the *y*-distance of younger plants to large founders was positively correlated with genetic similarity of younger plants to large founders might be explained by a chance effect. Differences in litter translocation distances and directions may have led to different amounts of seeds being introduced to the different mowing strips within the study area from adjacent portions of the population. In this scenario the observed negative relationship between *y*-distance and genetic distance of younger plants to large founders might arise if, for example, the mowing strip running through the spatial domain of the large founders had received a significant number of genetically dissimilar seeds whereas the adjacent, more distant strip had not.

In *B. orientalis* secondary seed dispersal due to anthropogenic disturbance appears to contribute to local genetic variability. Thus, if seeds are sampled locally, they will be genetically more variable in the presence than in the absence of such disturbance. As a consequence, if such seeds are transported to a previously uncolonized site, founder effects may be less pronounced.

Colonizing species frequently show low within- but high among-population genetic variation (Hamrick 1989) because of founder effects, geographic isolation, selection processes at new sites, and high selfing rates (Barrett and Shore 1989). However, for the colonizing annual *Raphanus raphanistrum* high within- and relatively low among-population variability has been reported from the northeastern USA (Kercher and Conner 1996). This was mainly attributed to long-distance dispersal by humans. Thus human impact may considerably affect the genetic structure of plants, both on an between-population scale as in the case of *R. raphanistrum*, and on a within-population scale as in our study of *B. orientalis*

Relationship between genetic variation and plant size

The low genetic variance among large founders suggests that these plants were more closely related to each other than were the smaller founders. Size in the field population was probably related to fitness because the genetic

distance of large founders to all possible descendants was lower than that of small founders to all possible descendants. In the case of *B. orientalis* fitness dominance of large founders may be mediated by polycarpy combined with carry-over of large size attained under favourable conditions after disturbance (cf. Dietz and Ullmann 1998).

Within the younger cohorts the larger plants were genetically less similar to large founders than were the smaller plants. Again, influences related to mowing and litter management may have caused this unexpected result. Within and near to the study region litter produced by mowing was left on the ground for several weeks before collection (I. Ullmann, personal communication). Compared with the other parts of the study area the patches exposed after removal of the litter dumps had probably received a higher number of translocated seeds and elevated resource levels because of reduced aboveground competition, higher moisture in the top soil and a substantial input of nutrients lost from the fresh cuttings (Schaffers et al. 1998). Thus, the plants recruited in these patches not only seem to have grown to larger sizes but also probably consisted of a higher proportion of genotypes imported to the study area, which may explain the relatively high genetic disparity between the large founders and large plants of younger cohorts.

Conclusions

The results of our study indicate that anthropogenic disturbance can have a considerable impact on the development of genetic structure within populations of *B. orientalis* by increasing gene flow and gene flow distances within (and between) populations. These effects may further genetic diversity and therefore weaken processes resulting from isolation by distance such as adaption to local environmental constraints or founder effects. A more general adaptability to unpredictable conditions at disturbed sites maintained in this way may be advantageous for the invasion process of many weeds because a wide array of introduced plant species are favoured by anthropogenic disturbance (e.g. Hobbs 1989; Dean et al. 1994; Kercher and Conner 1996).

The complexities of invasion processes have presented a major hurdle to the understanding of invasions in the past (Orians 1986). The combination of a census of the size and positions of plants with information on plant age obtained by herbchronology and on genetic variability obtained with RAPD-PCR allowed us to reveal the population history of a local invasion of *B. orientalis* over several years. Moreover, our *a posteriori* insights into microscale patterns of population development indicate the potential of our approach to generate testable hypotheses on the mechanisms affecting invasion success. For example, further experimental investigations should test (1) effects of typical management regimes on the development of spatial genetic structure during plant invasions on a local scale, and (2) whether genetic diversity of founder populations is related to average plant fitness in later stages of invasions.

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