Journal of Ecology

British Ecological Society

Journal of Ecology 2009, **97**, 131–141 doi: 10.1111/j.1365-2745.2008.01452.x

Temporal and spatial genetic variation in a metapopulation of the annual *Erysimum cheiranthoides* on stony river banks

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Summary

1. Metapopulation dynamics – the recurrent extinction and colonization in spatially discrete habitats – is expected to strongly affect within and between population genetic diversity. So far, however, accounts of true plant metapopulations are extremely scarce.

2. We monitored the colonization and extinction dynamics of an assemblage of populations of the annual *Erysimum cheiranthoides* on stony river banks during three consecutive years. Each year, winter flooding drives some populations to extinction, while vacant banks may become colonized. We describe the dynamics of these ephemeral populations using amplified fragment length polymorphism (AFLP) markers to quantify changes in the metapopulation genetic structure over time, and assessing the direction and relative amount of migration and colonization events.

3. Average extinction and colonization rates were high (0.39 and 0.34, respectively). While population genetic differentiation (F_{ST}) tripled from 0.06 in 2005 to 0.17 in 2007, total metapopulation genetic diversity remained fairly constant through the years. Genetic assignment analyses allowed assigning more than 50% of the genotyped individuals to populations extant the year before. Colonizing individuals originated from different source populations ($\varphi << 1$) and there was considerable evidence of upstream seed dispersal.

4. The degree and pattern of spatial genetic structure varied between years and was related to variation in the flooding intensity of the Meuse River through the years. Possibly, activation of the soil seed bank also played a role in structuring the genetic make-up of the populations.

5. Because migration and colonization events were qualitatively equal, and colonizing individuals originated from different sources, the increase in F_{ST} was in agreement with previous theoretical work. Very high migration and colonization rates, and the short monitoring period, may explain why there was no loss of genetic diversity from the metapopulation through recurrent extinction and colonization events.

6. *Synthesis.* This study gives one of the first accounts of the dynamics of a true plant metapopulation. Temporal monitoring of genetic variation gave evidence of extensive and bidirectional seed dispersal, highly variable and increasing genetic differentiation, and rather constant within population genetic diversity. An important suggestion from this research is to include a dormant seed stage in further theoretical work on (meta) population genetics.

Key-words: AFLP, assignment analysis, genetic differentiation, genetic drift, plant metapopulation, plant migration and colonization, riparian plant species

Introduction

Many plant populations consist of an assemblage of spatially discrete subpopulations connected by various amounts of seed exchange among them (e.g. Dupré & Ehrlén 2002; Soons & Heil 2002). These assemblages can be defined as metapopulations if they meet four criteria (Hanski 1999; Freckleton & Watkinson 2002): (i) the suitable subpopulation habitats are in spatially separated patches; (ii) all subpopulations can become extinct; (iii) recolonization of each patch after local

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extinction is possible; and (iv) not all subpopulations become locally extinct at the same time (i.e. the extinction dynamics are asynchronous). The main idea behind metapopulation theory is that if colonization rates are sufficient to offset local extinction, the metapopulation is expected to persist for a much longer time period than the constituent subpopulations (Hanski 1999). Although the extent to which plant species behave as metapopulations remains a point of discussion (Husband & Barrett 1996; Freckleton & Watkinson 2002; Ouborg & Eriksson 2004; Honnay *et al.* 2005), the metapopulation concept has at least proven to be an extremely fruitful heuristic framework to study the behaviour and fate of spatially subdivided populations. Accounts of assemblages of plant populations that strictly fit the four above-mentioned criteria are extremely scarce, however (Freckleton & Watkinson 2002).

One important line of theoretical research, initiated by Slatkin (1977), has focused on predicting the effects of recurrent extinction and colonization of suitable habitat patches on the within and between population genetic structure of the metapopulation (Pannell & Charlesworth 2000). Most of this work has focused on predicting the effects of metapopulation dynamics on genetic differentiation (F_{ST}) and has come to define under what conditions F_{ST} is expected to increase or decrease when the population is subject to recurrent local extinction and colonization events (Wade & McCauley 1988; Whitlock & McCauley 1990; Le Corre & Cremer 1998). The key to the response of F_{ST} was found to be the number of individuals colonizing empty patches (colonists) relative to the number of individuals migrating between extant populations (migrants), and the degree to which colonists are coming from different source populations (Pannell & Charlesworth 2000). When the number of colonists is small relative to the number of migrants, F_{ST} is expected to increase in comparison to the equilibrium case with no extinction–colonization dynamics. When the number of colonists is very large, F_{ST} is generally expected to decrease. The response to recurrent extinction and colonization of the total metapopulation effective size, and the within population genetic diversity has received somewhat less attention, although it is generally predicted that both will decrease due to founder events and genetic drift (Barton & Whitlock 1997; Pannell & Charlesworth 1999, 2000). These processes may also increase linkage disequilibrium (LD) between loci in subdivided populations (Ohta 1982; Zartman *et al.* 2006). Recently, genetic metapopulation models have also integrated other mediating variables such as life-history traits (Austerlitz *et al.* 2000) and the effects of long distance seed dispersal (Austerlitz & Garnier-Géré 2003; Bohrer *et al.* 2005). Given a certain local extinction rate, the discrimination between short and long distance seed dispersal has been shown to be crucial for understanding the genetic diversity and genetic structure of a plant metapopulation (Bohrer *et al.* 2005).

Theoretical work on the genetics of metapopulations has inspired empirical studies on plant species in at least two ways. First, the derived mathematical relations between the degree of genetic differentiation amongst populations and the relative number of colonists and migrants have been applied to infer the type and degree of regional population dynamics from population genetic snapshot data (e.g. Tero *et al.* 2003; DeWoody *et al.* 2004). Second, space-for-time substitutions (chronosequences) have been used to study the effects of colonization and founding events on population genetic differentiation (e.g. McCauley *et al.* 1995; Giles & Goudet 1997; Jacquemyn *et al.* 2004; Vandepitte *et al.* 2007). Because slow colonization and extinction dynamics in most plant species make it difficult to study plant metapopulations in real time (Ehrlén & Eriksson 2003), this chronosequence approach has been used as a substitute for temporal monitoring of genetic diversity. Clearly, this approach provides only partial information on the population genetic fate of a metapopulation as genetic diversity and differentiation among old and recently established populations are compared. Moreover, in many of these chronosequence studies there was no clear evidence of recurrent extinction and colonization.

We monitored genetic variation of a highly dynamic metapopulation of the annual plant species *Erysimum cheiranthoides* during three consecutive years (2005–2007). *Erysimum cheiranthoides* is a selfing pioneer species which is confined to sandy and stony habitats. The study system consisted of an assemblage of stony river banks along the only free flowing stretch of the River Meuse (E-Belgium) and fitted the above-mentioned metapopulation criteria rather well. Each year, winter flooding drives some populations to extinction, while vacant banks may become colonized. Extinction is highly stochastic and dependent on stream velocity variation which may locally wipe out a population independent of its size. Our general aims were to describe the population dynamics of *E. cheiranthoides* and to quantify temporal genetic variation during three consecutive years. More specifically, we applied Amplified Fragment Length Polymorphism (AFLP) markers to address the following questions:

1. Can the assemblage of *E. cheiranthoides* populations be qualified as a metapopulation in a strict sense?

2. During the studied time window, are genetic differentiation and genetic diversity decreasing or increasing as result of colonization–extinction dynamics?

3. Is there evidence of founder events and genetic drift in the populations through the occurrence of non-systematic LD between loci?

4. What are the direction, distance and the relative amount of migration and colonization events in the metapopulation?

Methods

STUDY AREA, SPECIES AND SAMPLING

The River Meuse is a rain-fed river, originating at an altitude of 409 m a.s.l. at the Plateau of Langres in the Northeast of France and discharging into the North Sea some 900 km further downstream. The Common Meuse, between Belgium in the West and The Netherlands in the East, is the 40 km long non-impounded, non-navigable reach of the River Meuse, where the river descends from the Ardennes and enters the lowlands. The high slope is responsible for its fast flowing gravel-bed river character. Discharge levels for the Common Meuse

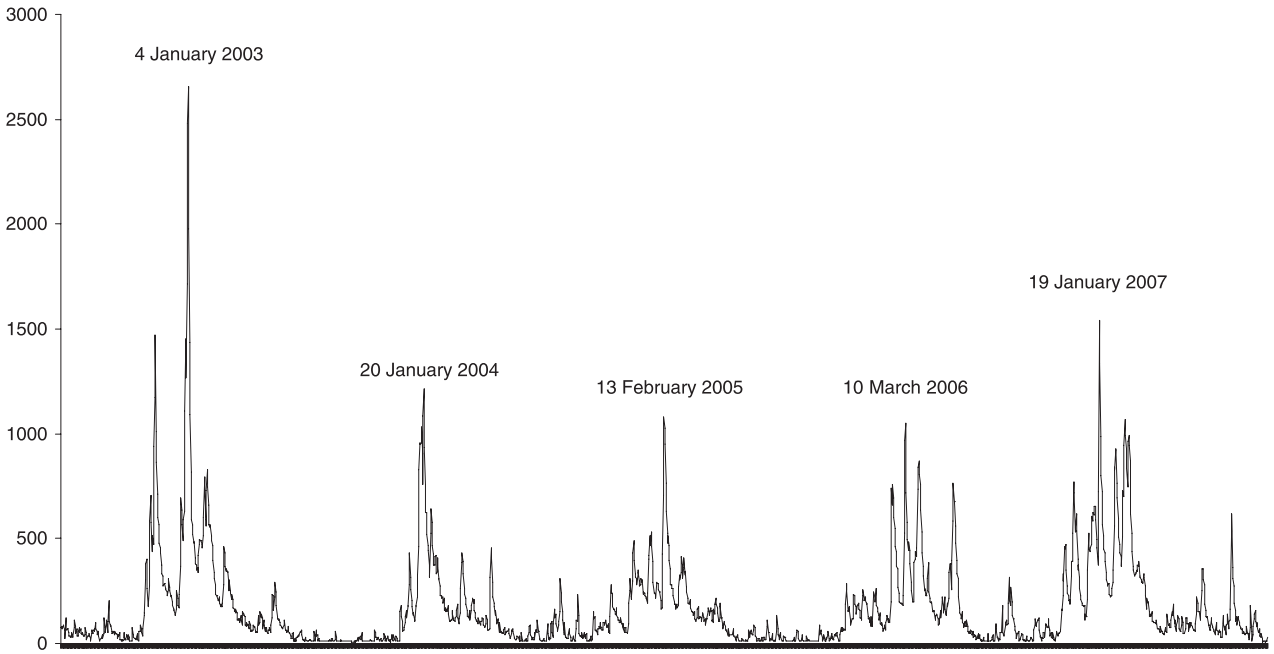

Fig. 1. Daily discharge ($m^3 s^{-1}$) through the free flowing stretch of the Meuse River between 1 November 2002 and 1 November 2007. Date of peak charges is indicated.

range from 10 $m^3 s^{-1}$ during dry periods to 10 000 $m^3 s^{-1}$ in periods of heavy rainfall in the catchment area.

Erysimum cheiranthoides L. (Treacle mustard or Worm seed) (Brassicaceae) is a short-rooted herbaceous annual species (15–60 (100) cm) with a Holarctic distribution (Hegi 1975). Its original habitat was very likely flooded river banks, but nowadays the species also occurs on arable land, road sides and in gardens. The bright yellow petals are 5–6 mm long, and flowers are produced in an erect inflorescence. The fruit consists of a cylindrical 1–3 cm long capsule, containing several small, dark brown seeds with no special adaptations for dispersal. The species is highly selfing, and in a glasshouse experiment seed set was observed after excluding pollen vectors (Nieto Feliner 1991). In our study area, the species is confined to stony river banks. Although the species already occurred in the study area before, the last large scale flooding (discharges > 2500 $m^3 s^{-1}$) dates back to January 2003 (Fig. 1). This resulted in a temporal connection with the high river banks of the upstream and strongly channelled stretch of the Meuse River and in the establishment of many populations on newly formed stony banks in the study area (K. Van Looy, personal observations). After January 2003, the inflow of seeds from the south into the study area has been very unlikely due to the absence of large floodings (Fig. 1), and we considered the studied metapopulation as a closed system.

Sixteen stony banks were located along the Common Meuse (Fig. 2). All banks are flooded during winter but the intensity of the water current strongly varies. This implies that all seeds are flushed away from some banks, leading to an empty patch the next year, while on other banks seeds remain in place to some extent. In September 2005, 2006 and 2007, all river banks were monitored for presence–absence of *E. cheiranthoides*. Population size of each extant population was determined by counting all individuals on the banks. For populations with more than 20 individuals, population size was estimated in 1–5 intervals. At the same time, a leaf sample was taken from each extant population for genetic analyses. We collected between 20 and 25 leaf samples per population. If the population size was smaller than 20 individuals, all individuals were sampled.

AFLP ANALYSES

All sampled leaves were immediately frozen in liquid nitrogen, freeze-dried for 48 h and homogenized with a mill (Retsch MM 200) to fine powder. DNA was extracted with the DNeasy Plant Minikit (Qiagen) from 20 mg of dried leaf material. DNA quality and concentration were estimated on 1.5% agarose gels. 100 ng of DNA were used for AFLP analysis according to Vos *et al.* (1995). Restriction and ligation were performed in a single step. Amplification of fragments was performed in two steps using the primer combinations EcoRI + AAG/MseI + CAT, EcoRI + AAG/MseI + CAG, EcoRI + AAG/MseI + CTA, EcoRI + ATC/MseI + CAC, EcoRI + ATC/MseI + CAT, EcoRI + ATC/MseI + CCA. Fragment separation and detection took place on a Nen IR^2 DNA analyzer (Licor) using 36 cm denaturing gels with 6.5% polyacrylamide. IRDye size standards (50–700 bp) were included for sizing of the fragments. Control samples were included in each gel to check for reproducibility between gels. The 2005 and 2006 samples were analyzed in a single assay, while the 2007 samples were processed 1 year later. The overall AFLP patterns were highly comparable (< 5% difference) between successive years. Only clear, intense bands were scored. Scoring was done using the SAGAmx software from Licor. We scored the presence or absence of every marker in each individual plant as 1 or 0 (present or absent) to form a binary data matrix.

DATA ANALYSIS

The following analyses were repeated for all populations in each sampling year separately. Total genetic diversity was partitioned among and within populations by carrying out a hierarchical analysis of molecular variance (AMOVA) on Euclidean pairwise genetic distances

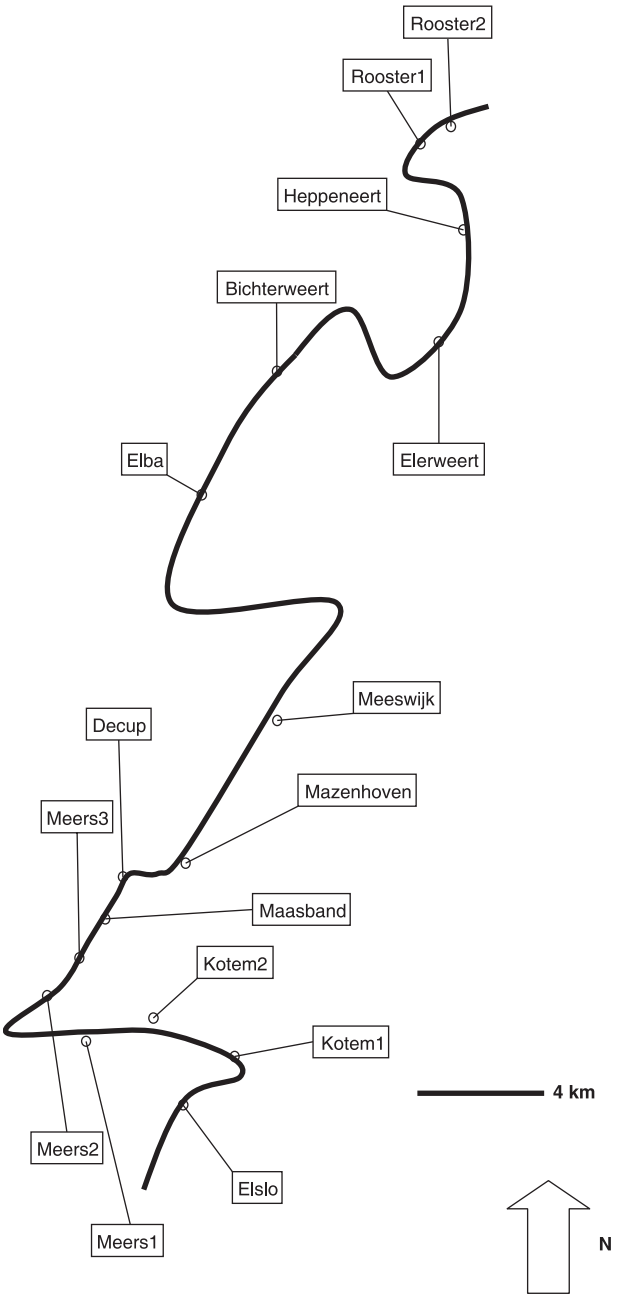

Fig. 2. Location of the 16 stony riverbanks along the Common Meuse. River discharge is from South to North.

(Huff *et al.* 1993), using GENALEX 6 (Peakall & Smouse 2006). The Φ_{ST} analogue for G_{ST} (Nei's coefficient of gene differentiation (Nei 1973)) was calculated based on Euclidian genetic distances, and its significance was determined using the Monte Carlo procedure available in GENALEX. AFLP allele frequencies for each population were estimated from the observed AFLP-fragment frequencies using the reliable square root method (Lynch & Milligan 1994; Bonin *et al.* 2007). Allele frequencies were then used to calculate expected heterozygosity (H_j), percentage of polymorphic loci (PPL), proportion of the total genetic variance contained in a subpopulation relative to the total genetic variance (F_{ST}) and total metapopulation diversity (H_t) (Lynch & Milligan 1994). These calculations were carried out using AFLP–SURV (Vekemans *et al.* 2002) and require prior knowledge of the population inbreeding coefficient (F_{IS}). F_{IS} of each population was obtained by averaging the inbreeding coefficient f of each individual, calculated according to the method of Dasmahapatra *et al.* (2007).

Where common population genetic diversity measures are confined to allele frequencies at a single locus, the degree of association between alleles at different loci can also be informative (Weir 1979). Increased linkage disequilibrium (LD), or the non-random association between pairs of loci, has, for example, been observed in fragmented epiphyll colonies compared to colonies in continuous habitat (Zartman *et al.* 2006). We used a χ^2 test to calculate the number of significant associations between loci in each population. We then calculated Ohta's D-statistics, available in POPGENE 1.31, to partition the variance in LD into within (D^2_{ST}, D'^2_{ST}) and between (D^2_{ST}; D'^2_{ST}) population components (Ohta 1982), and to detect whether LD was systematic or not systematic in each population. In the latter case LD is attributable to non-selective forces of population subdivision or founder events (Ohta 1982; Volis *et al.* 2001). Every sample was considered as a haploid gamete.

For each separate year, spatial genetic structure of the populations was examined using a Mantel test, performed with GENALEX 6. The Mantel test was performed on the triangular matrix with pairwise geographical distances between populations and the triangular matrix with pairwise F_{ST} values between populations. The latter were calculated using AFLP–SURV with the F_{IS} values calculated as indicated above. We also constructed an UPGMA dendrogram based on Nei's genetic distance for all populations over all years (POPGENE 1.31). In order to identify a possible trend in genetic variation along the river course (due to mainly downstream seed dispersal (Ritland 1988, Markwith & Scanlon 2007)), a Spearman rank correlation was calculated between H_j and PPL and the rank order of the populations along the river. Finally, and in order to partition total metapopulation genetic variance between populations and between years, we performed a hierarchical AMOVA in GENALEX 6.

In order to identify colonization and migration events among populations and between years, we finally assigned all genotyped individuals of 2007 to the 2006 populations and all 2006 individuals to the 2005 populations. We used the likelihood based assignment test implemented in AFLPOP (Duchesne & Bernatchez 2002), which incorporates an adaptation of the method of Paetkau *et al.* (1995) for dominant AFLP markers. The advantage of this approach is that no complete sampling of all extant individuals is required (Manel *et al.* 2005). The minimal log-likelihood difference for the allocation of an individual to a population was set at 0.5. To test for the closed nature of the metapopulation, we also performed simulations to determine the number of individuals which may be immigrants from outside the metapopulations, either within or between years, as outlined in He *et al.* (2004).

Results

Extinction rates of the species were 0.50 (2005–2006) and 0.29 (2006–2007); colonization rates 0.14 (2005–2006) and 0.53 (2006–2007). Population size over all banks and over the 3 years averaged 38 individuals (range 1–210) (Table 1). Patch occupancies of the species were 0.75 (2005), 0.44 (2006) and 0.81 (2007) (Table 2).

The six AFLP primer combinations resulted in 67 highly reliable polymorphic markers. Our data set contained information for 213 individuals in 2005; 95 in 2006 and 220 in 2007 (total 528 samples). In one population ($Mazenhoven_{2007}$)

Table 1. Characteristics of 16 *Erysimum cheiranthoides* populations on stony banks along the Meuse River, monitored during three consecutive years. n, number of individuals; H_j, expected heterozygosity; PPL, percentage polymorphic loci; –, missing data. x and y coordinate according to Belgian Lambert grid.

	x†	y	n_{2005}	n_{2006}	n_{2007}	$H_{j\,2005}$	$H_{j\,2006}$	$H_{j\,2007}$	PPL_{2005}	PPL_{2006}	PPL_{2007}
Elslo	246 880	182 675	13	45	210	0.194	0.238	0.219	55.2	64.2	53.7
Kotem1	247 561	183 488	0	0	25			0.241			68.7
Kotem2	246 486	184 131	20	0	200	0.216		0.219	56.7		52.2
Meers1	245 594	183 743	50	100	0	0.178	0.242		47.8	64.2	
Meers2	245 079	184 508	100	9	50	0.252	0.271	0.229	62.7	64.2	58.2
Meers3	245 505	185 142	25	0	0	0.198			49.3		
Maasband	245 849	185 796	0	0	35			0.23			59.7
Decup	246 084	186 503	25	50	150	0.218	0.244	0.215	62.7	65.7	56.7
Mazenhoven	246 913	186 730	14	0	1	0.210		–	53.7		–
Meeswijk	248 126	189 125	18	14	20	0.229	–	0.216	65.7	–	62.7
Elba	247 126	192 912	0	40	25		0.240	0.182		67.2	49.3
Bichterweert	248 122	194 985	0	0	25			0.190			58.2
Elerweert	250 265	195 481	100	0	35	0.209		–	52.2		–
Heppeneert	250 592	197 359	50	0	25	0.201		0.297	55.2		73.1
Rooster1	250 022	198 804	155	0	65	0.228		0.212	56.7		47.8
Rooster2	250 426	199 092	100	13	0	0.228	0.242		58.2	64.2	
Average (SD)			42	17	57	0.213a* (0.020)	0.246b (0.012)	0.223ab (0.030)	56.3a* (5.4)	64.9b (1.3)	58.2ab (7.8)

*Letters indicate significant ($P < 0.05$) differences between years according to a Kruskal–Wallis test.

Table 2. Average metapopulation characteristics by year. H_t, total metapopulation genetic diversity; F_{ST}, genetic differentiation; LD, percentage of loci with linkage disequilibrium; r, Mantel test statistic for isolation by distance (**$P < 0.001$)

Year	Patch occupancy	Total number of individuals	Number of occupied patches	River peak discharge ($m^3\ s^{-1}$)	H_t	F_{ST}	LD (%)	r
2005	0.75	670	12	1078	0.227	0.06	19	0.02
2006	0.44	271	7	1109	0.277	0.11	13	–0.09
2007	0.81	866	12	1679	0.269	0.17	23	0.68**

only one individual was present which was omitted from all analyses. In two other populations no genetic data could be retrieved ($Meeswijk_{2006}$, $Elerweert_{2007}$).

Average genetic diversity (both H_j and PPL) of the populations was significantly higher in 2006 than in 2005, with an intermediate position for 2007 (Table 1). Variability of H_j among years and among populations was relatively low (Fig. 3). For none of the 3 years, the Spearman rank correlation between the rank order of a population along the river and PPL and H_j was significant. This implies that genetic diversity is not accumulating in the more downstream populations, impoverishing upstream populations.

Total metapopulation genetic diversity (H_t) was 0.227, 0.277 and 0.269 for 2005, 2006 and 2007, respectively (Table 2). There was no homozygosity excess and F_{IS} averaged over all populations ranged from 0.00 (2005) over 0.01 (2006) to –0.03 (2007). All F_{ST} values were significantly different from 0 ($P < 0.001$), and increased from 0.06 (2005) over 0.11 (2006) to 0.17 (2007) (Table 2). Values were significantly different between years (bootstrapped 95% confidence interval). AMOVA-derived Φ_{ST} values were very similar to F_{ST} (0.06 (2005), 0.08 (2006) and 0.18 (2007)) and were also significantly different from 0 ($P < 0.001$).

In 2005, on average 19% of all loci showed a significant LD; in 2006 this was 13%, and in 2007 23%. This is considerably higher than the 5% expected by chance. The different variance components of LD were compared and for each year: both ($D'^2_{ST} >> D'^2_{ST}$) and ($D^2_{ST} >> D^2_{ST}$) (results not shown). This implies that the observed LD is not systematic and is likely to be caused by drift.

The Mantel tests revealed significant isolation by distance in 2007 ($r = 0.68$; $P = 0.01$), but not in 2005 ($r = 0.02$; $P = 0.40$) and 2006 ($r = –0.09$; $P = 0.66$) (Fig. 4). The UPGMA dendrogram (Fig. 5) shows a remarkable clustering of the 2007 populations and confirms that geographical structuring is very weak in 2005 and 2006, while some geographical structuring is present in 2007. The hierarchical AMOVA on all 528 samples revealed that 10% of the variation was due to variation between banks and 12% was due to variation between years.

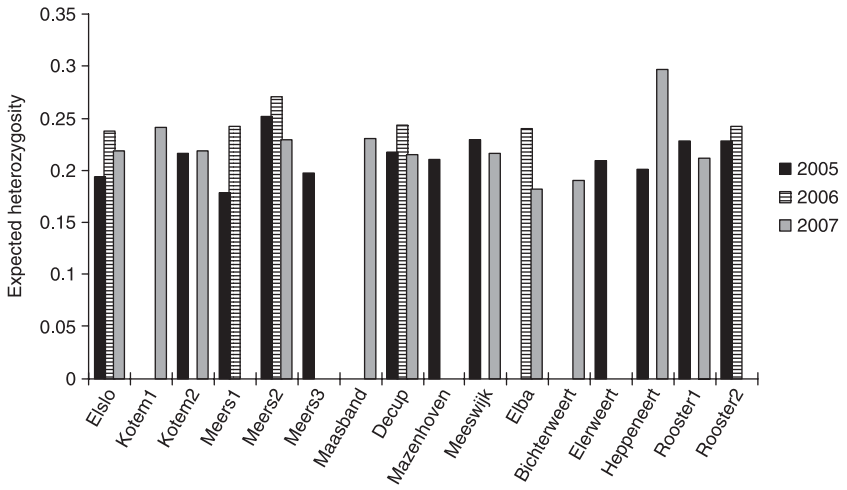

Fig. 3. Expected heterozygosity (H_j) of 16 populations of *Erysimum cheiranthoides* during the three consecutive years of monitoring. The populations are ordered according to their position along the river in the downstream direction, that is, from South to North.

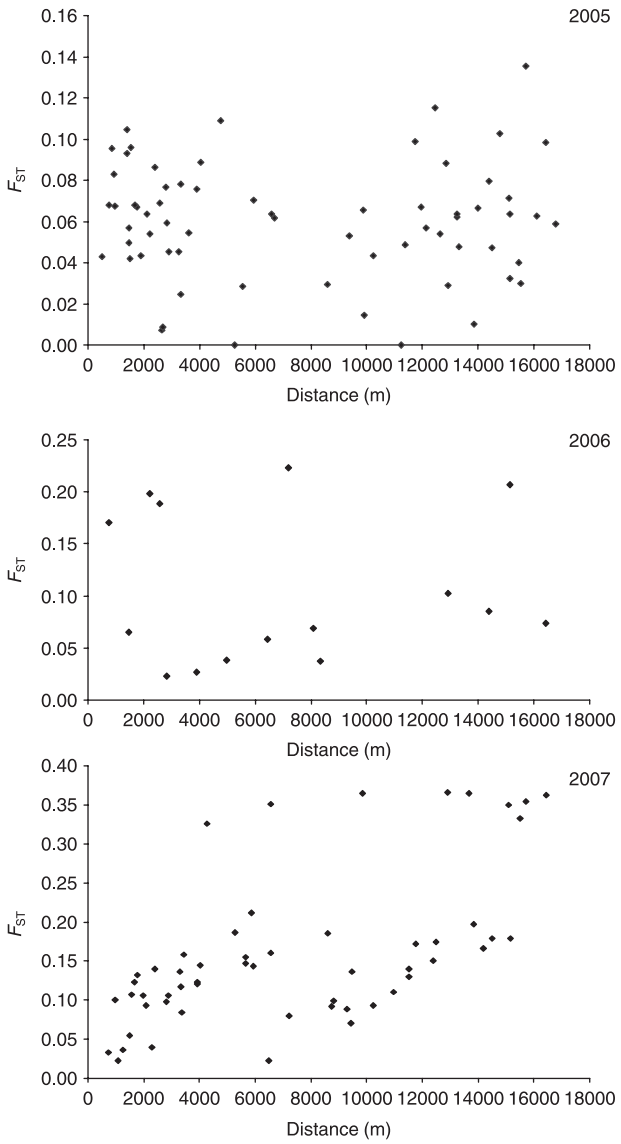

Fig. 4. Isolation by distance relations in a metapopulation of *Erysimum cheiranthoides* during three consecutive years.

Fifty percentage of all individuals sampled in 2006 could be assigned to populations extant in 2005 and 58% of the 2007 individuals could be assigned to 2006 populations (Table 3). In 2006 there were eight source populations involved, in 2007 only four. In 2006, we identified the sources of 9 colonists and 40 migrants, and in 2007 of 52 colonists and 68 migrants. The majority of all dispersal events took place downstream, although a considerable number occur upstream (Table 3). Of the 2006 individuals only two could not be assigned to a sampled population and of the 2007 individuals only 12 could not be assigned. These results strongly suggest that the studied metapopulation is a closed system. It cannot be completely excluded, however, that some very small populations on small stony parts of the river bank were not inventoried.

Discussion

GENETIC DIFFERENTIATION IN A PLANT METAPOPULATION

The studied assemblage of *E. cheiranthoides* populations on 16 stony river banks along the free flowing stretch of the Meuse River clearly exhibited metapopulation dynamics in the strict sense. The conditions of asynchronous extinction, discrete habitat patches, and chances of extinction and colonization being similar for all banks, irrespective of their spatial location, were all met. Therefore, this study system is one of the rare accounts of a true plant metapopulation in the strict sense (Freckleton & Watkinson 2002). Because of the high extinction and colonization rates and the large amounts of seed flow between extant populations and towards vacant banks, the metapopulation was also highly dynamic.

We found a remarkable increase in genetic differentiation between the populations over 3 years of monitoring (F_{ST} increased from 0.06 in 2005 to 0.17 in 2007), whereas there was no evidence of decreasing genetic diversity, neither at the population nor the metapopulation level. F_{ST} values reported in other studies of dynamic riparian plant populations fall

Table 3. Assignment of individuals to populations extant the year before. Colonists and recolonized banks are in italic, migrants are in bold

	2006 individuals assigned to 2005 populations						2007 individuals assigned to 2006 populations										
Source↓	Elslo	Meers1	Meers2	Decup	*Elba*	Rooster2	Elslo	*Kotem1*	*Kotem2*	Meers2	*Maasband*	Decup	Meeswijk	Elba	*Bichterweert*	*Heppeneert*	*Rooster1*
Elslo	**1**				*2*												
Kotem1																	
Kotem2	**2**	**1**				**1**											
Meers1		**3**				**1**	**7**	*5*	*11*	**13**	*6*	**6**	**7**	**5**	*5*	*2*	*1*
Meers2				**2**													
Meers3	**2**				*2*												
Maasband																	
Decup	**1**	**1**	**2**			**1**	**2**	*4*	*2*	**2**		**5**	**9**	**1**		*2*	*3*
Mazenhoven																	
Meeswijk																	
Elba							**2**			**1**	*5*	**2**	**1**	**4**		*5*	*2*
Bichterweert																	
Elerweert	**1**			**1**													
Heppeneert	**2**	**2**			*1*												
Rooster1			**1**													*2*	
Rooster2	**2**	**2**	**1**	**6**	*4*	**1**	**1**				*3*						
Assigned	0.61	0.60	0.44	0.50	0.45	0.40	0.50	0.39	0.54	0.84	0.70	0.65	0.85	0.50	0.25	0.61	0.55

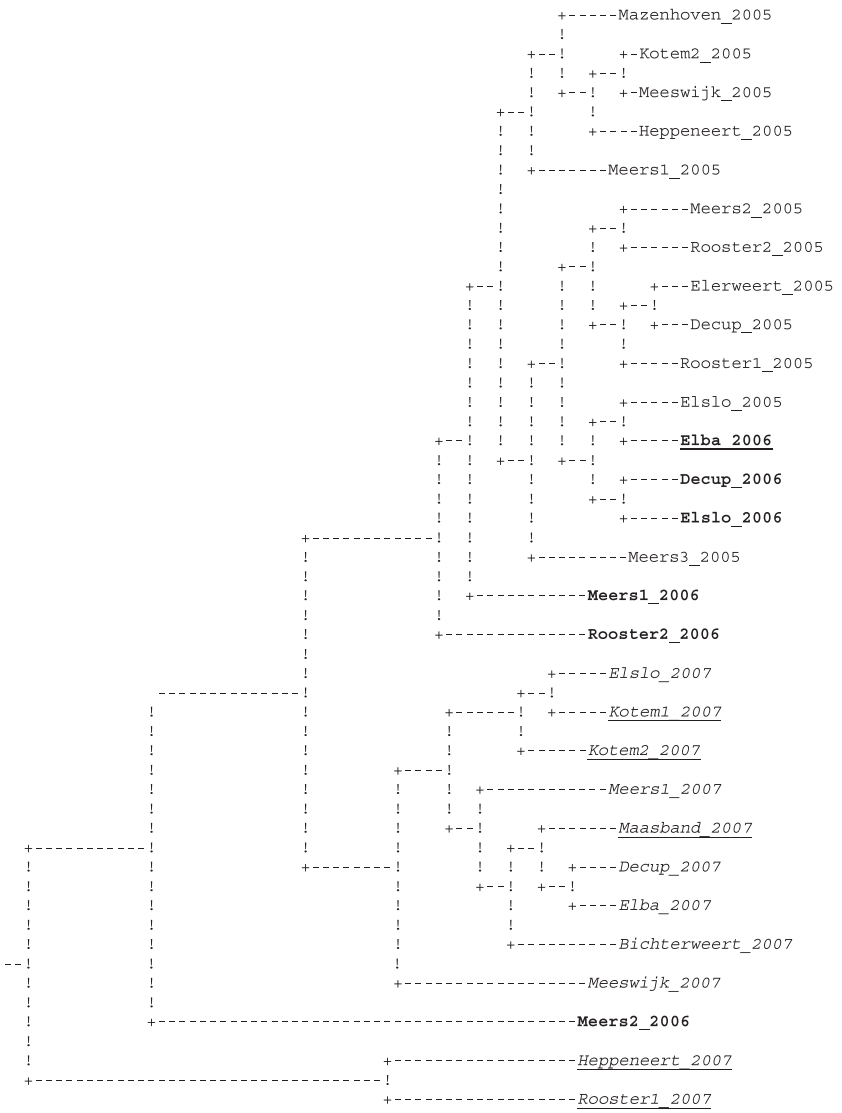

Fig. 5. UPGMA dendrogram of all populations of *Erysimum cheiranthoides* based on Nei's unbiased genetic distance. Different fonts for different years were used. Colonizations are underlined.

within the range of the values we found (0.098 in *Boltonia decurrens* (DeWoody *et al.* 2004); 0.097 in *Sisymbrium austriacum* in the same river system (Jacquemyn *et al.* 2006) and 0.062 in *Hibiscus moscheutos* (Kudoh & Whigham 1997). Tero *et al.* (2003) found higher genetic differentiation (0.287) in *Silene tatarica*. The observed high variability of genetic differentiation between years, however, strongly questions the reliability of snapshot F_{ST} data to infer regional population dynamics, especially in very dynamic river systems. Therefore, comparison of F_{ST} values between river systems, and even between species within river systems seems very difficult.

The genetic structure of plant metapopulations is not only influenced by seed dispersal but also by pollen flow between populations (McCauley *et al.* 2001). Given the considerable distance between the stony banks in our study system (average 7290 m; median 6340 m; minimum 496 m), we consider gene flow through pollen between extant populations as virtually absent. Besides, previous studies (Nieto Feliner 1991) have shown that the species is able to self, thus restricting pollen flow. Whitlock & McCauley (1990) have then shown that F_{ST} increases as a result of recurrent extinction if:

$$k < 2Nm/(1 - \varphi) + 0.5$$

where k is the number of colonists in the metapopulation, Nm the number of migrants, and φ the probability that two colonists come from the same source population. In case $\varphi = 1$ (the so called *propagule pool* model (Slatkin 1977)), colonization (i.e. the establishment of a population on an empty bank) will always increase F_{ST}. Based on the results of the assignment analyses, however, this scenario can be rejected. With the exception of the Bichterweert$_{2007}$ population, the recolonized banks contained individuals originating from at least two and maximal four different source populations. The number of colonizing individuals originating from a single source population varied between 1 and 11 (average 4), and suggests that colonization generally happens through dispersal of whole fruits or even whole plants, originating from a very

restricted number of different source populations (McCauley *et al.* 2001). The colonization mode in our system is therefore intermediate between the migrant pool model ($\varphi = 0$; Slatkin 1977) and the propagule pool model. Abstraction of the number of individuals that could not be assigned unambiguously, the probability of two randomly chosen colonists originating from the same source population can be calculated based on the data in Table 2, using simple combinations of probabilities. We find $\varphi_{2006} = 0.22$ and $\varphi_{2007} = 0.44$, values considerably lower than the ones reported by McCauley *et al.* (1995) in founder populations of *Silene alba*. This implies that, given that the number of assigned colonists and migrants is of the same order of magnitude (Table 2), φ is sufficiently high to allow k to be smaller than $2Nm/(1 - \varphi) + 0.5$. Although the high number of unassigned migrants does not allow providing an accurate assessment of Nm, our results seem to confirm the theoretically expected increase of F_{ST} compared to the case where extinction–colonization dynamics are absent (Pannell & Charlesworth 2000).

Pronounced differences in probability of common origin of propagules between years may be related to the higher flooding intensity in 2007 compared to 2006 (Fig. 1). Higher φ, in turn, seems to be in accordance with the more pronounced spatial genetic structure in the 2007 metapopulation than in the 2006 metapopulation. Although we cannot establish a general relation between flooding intensity and spatial genetic structure because our data covers only 3 years, our study illustrates the potential complex interaction between seed dispersal and metapopulation genetic structure and urges for the collection of more long-term data.

Bohrer *et al.* (2005) have recently modelled the effects of long-distance seed dispersal on the genetic structure of a linear metapopulation. They showed that migration of individuals among populations has a homogenizing effect on the metapopulation structure, while colonization of empty patches has a subdividing effect. Bohrer *et al.* (2005) also showed that the subdividing effect of seed dispersal prevails under high extinction probabilities. Because the extinction rates we measured in the *E. cheiranthoides* populations are two to five times higher than their highest modelled extinction rates, our results seem to support their general expectations that F_{ST} increases among ephemeral populations, connected by long distance seed dispersal.

METAPOPULATION AND POPULATION GENETIC DIVERSITY

Genetic diversity within the studied populations was rather constant throughout 3 years of monitoring. Compared to the average expected heterozygosity of annual selfing species, diversity was also fairly high. Nybom & Bartish (2000) reported average RAPD-based expected heterozygosities of 0.219 and 0.091 for species with a mixed breeding system and for selfing species, respectively. The combination of observed high genetic diversity with the absence of a homozygosity excess for each of the three survey years, suggests that, although in the glasshouse the flowers of *E. cheiranthoides* do not rely on pollen vectors to accomplish seed set (Nieto Feliner 1990), in natural environments outcrossing may be frequent in this species.

Contrary to theoretical expectations (Barton & Whitlock 1997, Pannell & Charlesworth 1999) we found no evidence of declining metapopulation or population genetic diversity. Wang & Caballero (1999) have demonstrated that extinction–colonization dynamics lower effective population size (Ne) of a metapopulation – increasing the chance of random loss of genetic variation – while the effect of population subdivision per se depends on the variance in reproductive success among individuals in the metapopulation. If individuals from different populations unequally contribute to offspring (due to qualitative differences between populations or simply because populations are on the brink of extinction) N_e is expected to decrease (Wang & Caballero 1999), increasing the rate of decay of neutral genetic variation. Very high migration rates, as observed in the study species, can, however, offset these variances in reproductive output. Moreover, the age of the studied metapopulation, originating after the January 2003 flooding, was less than 5 years. As F_{ST} is directly affected by the redistribution of genetic variation over the different subpopulations, it responds quickly to extinction and recolonization, whereas absolute genetic diversity measures react more slowly because they are dependent on a process such as drift (Pannell & Charlesworth 1999). The moderate levels of LD on the other hand suggest some degree of random genetic drift in the studied metapopulation, a phenomenon also reported in subdivided populations of vascular plants (Tero et al. 2003), bryophytes (Zartman et al. 2006), watervoles (Stewart et al. 1999) and parasites (Barriere & Felix 2007).

SPATIAL GENETIC STRUCTURE AND DIRECTION OF DISPERSAL

A considerable number of individuals of the populations extant in 2006 and 2007 were assigned to downstream populations, giving evidence for upstream seed dispersal. Although contra-intuitive, others have reported upstream seed dispersal in riparian plants as well (Tero *et al.* 2003; DeWoody *et al.* 2004). The observation of upstream seed dispersal is in accordance with the absence of a rank correlation between the location of a population along the river course and its genetic diversity, and refutes the hypothesis of unidirectional gene dispersal, which states that because seed dispersal is mainly downstream, effective population size and genetic diversity should be higher in the downstream parts of river systems (Ritland 1988). Markwith & Scanlon (2007) also did not find accumulation of genetic diversity in downstream populations of the macrophyte *Hymenocallis coronaria*. Potential vectors for upstream seed dispersal are (water)birds that forage on the stony banks and use the river as a migration corridor (see, e.g. Soons *et al.* 2008). Additionally, amateur fisherman may transport seeds between the banks.

The absence of geographical genetic structure in 2005 and 2006 indicates that seed dispersal is not more likely between adjacent banks, and that long distance seed dispersal is very

important, which was also reported by Jacquemyn *et al.* (2006) for the riparian plant species *S. austriacum* in the same study region. Even the most remote downstream banks as well as upstream banks have a good chance of becoming colonized which confirms the true metapopulation structure of the study system. Following the colonization events during winter 2007, however, some geographical structure seems to have emerged within the 2007 populations. Remarkable is that most of the 2007 populations cluster together. Whereas the 2006 flooding was relatively weak, comparable with the floodings of 2004 and 2005, the 2007 flooding was more intensive (Fig. 1). This may have caused the many colonization events observed between 2006 and 2007 (colonization rate$_{2006–2007}$ = 0.53) and may have resulted in a complete rearrangement of the genetic make-up of the populations due to large numbers of seeds that were dispersed between river banks.

Additionally, it is not impossible that a buried seed bank was activated during the moderate 2007 flooding. Local activation of a seed bank (seed dispersal through time) may also have offset the random loss of genetic variation in the study species, providing an additional explanation why metapopulation genetic diversity has not decreased through 3 years of monitoring. Persistent seed banks have indeed been shown to be able to mitigate loss of genetic variation through drift (Honnay *et al.* 2008) and the seeds of *E. cheiranthoides* remain viable in the soil for 5 years or more (Thompson *et al.* 1997).

Even without the seed bank complication, our study highlights a conceptual problem regarding the definition of 'colonization' and 'migration' in an annual plant species. When a population persists from one year to the next, the incoming individuals from elsewhere are by definition classified as migration events, even when no local individuals produced offspring (all local genotypes went extinct), and the persistence of the population is completely due to colonization from other sources. The distinction between migration and colonization therefore seems ambiguous and a more accurate description of population continuity is required in annual species.

Conclusion

We found sufficient evidence to conclude that the assemblage of populations of *E. cheiranthoides* on 16 stony river banks along the free flowing stretch of the Meuse River behaves as a very dynamic metapopulation in the strict sense. Therefore this study provides some of the few examples of a true plant metapopulation. Temporal monitoring of genetic variation in this metapopulation showed evidence of extensive and bidirectional seed dispersal, highly variable and increasing genetic differentiation and rather constant within population genetic diversity. High exchange rates of individuals between banks and the relatively recent origin of the metapopulation may explain why recurrent extinction and colonization have not caused a decrease of genetic diversity. Additionally, we cannot exclude a role of the persistent seed bank, both in maintaining genetic diversity and in structuring the population after the moderate flooding event of 2007. Husband & Barrett (1996) already noticed that the assessment of extinction rates is straightforward in animal species, but far more complicated in plant species with persistent seed banks. We can re-address their question whether (partial) re-emergence of a population from the seed bank should be considered as colonization in time, analogous to colonization in space, and urge for the integration of a seed bank stage in genetic (meta)population models (see, e.g. Vitalis *et al.* 2004).

Acknowledgements

This research was funded by the Flemish Fund for Scientific research (FWO project G.0310.05N). HJ holds a postdoctoral fellowship from FWO. KV is supported by IWT-Flanders. Two anonymous referees provided thoughtful comments on an earlier manuscript. Thanks to Nancy Van Liefferinge (INBO) for laboratory assistance.

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Received 7 July 2008; accepted 26 September 2008
Handling Editor: Joop Ouborg