Journal of Ecology 2003
91, 578–586

Spatial patterns of maternal lineages and clones of *Galium odoratum* in a large ancient woodland: inferences about seedling recruitment

BIRGIT ZIEGENHAGEN*, RONALD BIALOZYT*, VIVIAN KUHLENKAMP*, INGE SCHULZE*, ANDREAS ULRICH† and MONIKA WULF‡

**Federal Research Centre for Forestry and Forest Products, Institute for Forest Genetics and Forest Tree Breeding, Sieker Landstrasse 2, D-22927 Grosshansdorf, Germany, †Institute of Primary Production and Microbial Ecology, Centre for Agricultural Landscape and Land Use Research, Eberswalder Strasse 84, D-15374 Müncheberg, Germany, and ‡Institute of Land Use Systems and Landscape Ecology, Centre for Agricultural Landscape and Land Use Research, Eberswalder Strasse 84, D-15374 Müncheberg, Germany*

Summary

1 We investigated spatial genetic patterns in the clonal herbaceous plant species *Galium odoratum* L., which has previously been shown to be significantly associated with ancient woodlands.

2 A maternally inherited chloroplast DNA marker was developed for *G. odoratum*. Seven chloroplast haplotypes were detected in the study area and mapped at different spatial scales. Their spatial patterns suggest that both local and non-local gene flow occurs via seeds.

3 Fingerprints from DIG-labelled AFLP technology were validated as markers for *G. odoratum* clone identification. A single primer–enzyme combination was sufficient to distinguish between clones. A typical patch of *G. odoratum* is composed of numerous genets, mainly occurring in separate clusters of ramets, which may be several meters in diameter.

4 Pairwise similarities, calculated from the AFLP banding patterns, were used for spatial autocorrelation analysis. The distinct spatial genetic structure at the patch level may be due to the genetic similarity of neighbouring genets or to clonal growth.

5 The spatial patterns obtained using the two DNA markers, suggest that a mixture of local and non-local gene flow via both seeds and pollen is occurring. Repeated seedling recruitment (RSR) would be consistent with the life history of *G. odoratum* in ancient woodlands with long-term spatial continuity.

Key-words: AFLP, clone size, cpDNA polymorphism, seed dispersal, spatial autocorrelation

Journal of Ecology (2003) **91**, 578–586

Introduction

The spatio-temporal continuity of wooded areas can be monitored by comparing historical and current maps. So-called ancient woodlands in Great Britain can be traced back for at least 400 years (Peterken 1981) and for at least 250 years in Central Europe (Wulf 1994). Numerous floristic studies in several European countries suggest that certain vascular plant species are indicative of ancient woodlands, including the rhizomatous clonal herb *Galium odoratum* L. (e.g. Rackham 1980; Brunet 1994; Wulf 1997). It is not however, clear whether the distribution of the species is due to poor dispersal ability or to other ecological factors. Clonal migration (i.e. rhizome growth) averaging 20 cm year^{-1} (Fischer 1987; Lippert-Hambacher 1992) and up to 40–100 cm year^{-1} (Lippert-Hambacher 1992; Petersen & Philipp 2001) has been reported.

§Present address and correspondence: Birgit Ziegenhagen, Philipps-University of Marburg, Faculty of Biology, Nature Conservation Division, Karl-von-Frisch-Strasse, 35032 Marburg, Germany (tel. + 49 6421 2826585, fax +49 6421 2826588, e-mail ziegenha@staff.uni-marburg.de).

© 2003 British Ecological Society

Following the method of Matlack (1994), Brunet & von Oheimb (1998) calculated similarly low migration rates (20 cm year^{-1}) from the above-ground coverage of plots along a transect from ancient into adjacent recent woodland. However, a few *G. odoratum* individuals were found further from the migration front. Although long-distance dispersal of the adhesive seeds by animals is possible, *G. odoratum* seeds were rarely recovered from the fur of wild boar and dogs (Luftensteiner 1982; Heinken 2000). Thus, many questions related to the efficiency of seed dispersal remain unanswered. Nor is the spatial structure of clones known, so that patch formation could be mainly due to vegetative propagation or could involve repeated seedling recruitment (RSR) (Eriksson 1993). Because these processes are supposed to be mainly stochastic, we would still lack information about selective processes. For example, it has been reported that in *Galium boreale* L., a clonal species native to England and Wales, the recruitment of specific individuals is dependent on habitat (Dale & Elkington 1984).

We applied genetic markers as an indirect method of inferring seed dispersal and clonal spread in *G. odoratum*. Using neutral markers, we focused on stochastic aspects of the spatial distribution of seedlings and clones. This was because of the hypothesis that the failure to colonize recent woodlands is more due to the low dispersal ability than to habitat conditions (e.g. Brunet *et al*. 2000). Our goal was to obtain knowledge on species-specific dispersal modes and gene flow under most natural conditions. Consequently, the study was done in an ancient woodland with great spatio-temporal continuity. In the discussion we considered whether the dispersal modes or other ecological factors may restrict migration or colonization of recent woodlands.

Materials and methods

STUDY AREA

The Grumsin Forest, located about 50 km north of Berlin, Germany (53°00′ N, 13°50′ E), is a large non-fragmented ancient woodland covering about 1400 ha and dating back at least to the 16th century. The woodland communities belong to the phytosociological class of *Querco-Fagetea* (Ellenberg *et al*. 1992) and about 70% of the area is beech forest. Soils range from relatively acidic and more or less dry soils poor in nutrients (*Milio-Fagetum*) to slightly acidic, fresh soils with a moderate level of nutrients (*Melico-Fagetum*). The area is divided into about 80 forest management sections with an average size of 15–20 ha (Fig. 1) and about 40 of these contain patches of *G. odoratum* typically covering a few square metres.

PLANT MATERIAL FOR DETECTION OF CHLOROPLAST DNA POLYMORPHISM AND VALIDATION OF AFLP FINGERPRINTS

Chloroplast (cp) DNA polymorphism had not been described in *G. odoratum* prior to this study. In order to increase the probability of detecting cpDNA variants, five sprouts were sampled from each of five areas: an ancient woodland east of Hamburg, Germany

Fig. 1 Map of the Grumsin Forest and spatial distribution of the seven *G. odoratum* chloroplast haplotypes using TF–*Hinf* I (Fig. 2). *Galium odoratum* occurs in the white areas of the map. Intensive sampling was undertaken in a 10 × 400 m rectangle on the northern edge and in a large isolated patch (asterisk). Groups of three symbols in the rectangular patch represent the three samples from each single plot.

© 2003 British Ecological Society,

(53°44′ N, 10°27′ E), three small ancient woodlands north west of Berlin, Germany (53°16′ N, 12°12′ E; 53°17′ N, 12°01′ E; 53°17′ N, 12°03′ E), and the study area. Maximum distance between the five woodlands was about 250 km, and minimum distance between the five sprouts was about 50 m.

In order to validate AFLP patterns as fingerprint markers, two neighbouring patches in the woodland near to Hamburg were sampled, the two being about 2 m apart. From each patch, four sprouts were sampled that were either direct neighbours or at maximum distance of about 50 cm from each other. Three different AFLP primer–enzyme combinations were compared for their power to discriminate clones. For testing the reproducibility of AFLP patterns, the same sprout was sampled from two different whorls.

SAMPLING STRATEGY FOR SPATIAL GENETIC ANALYSIS

Sampling was performed at three different spatial scales: the whole study area (i.e. all 40 sections in 'Grumsin Forest' containing *G. odoratum*, Fig. 1), within a 400 m long rectangle (400 × 10 m) in a section on the northern edge of the forest (Fig. 1), and in an isolated patch (5 × 10 m) towards the south of the forest (Fig. 1). The cpDNA marker variation was analysed at all three scales, the AFLP markers only at the two smaller scales.

Throughout the whole study area, up to four neighbouring sprouts were sampled. Altogether, 59 sprouts were sampled.

Within the 400 × 10 m rectangle, eight small plots were sampled. These were located about 50–60 m apart and were mainly disjunct patches covering an area of 1–3 m^2. Three sprouts were randomly sampled from each plot, resulting in a total of 24 samples.

The single large patch was sampled using a grid design. Two different levels of spatial resolution were considered: the whole patch was sampled at 1.25 m distance intervals and the densely colonized, central part of the patch was sampled at 0.50 m intervals (the 'zoom-in' patch). A total of 66 samples were analysed.

DNA EXTRACTION

DNA was extracted from 50 mg to 100 mg (fresh weight) leaf material of each sample, following either a CTAB-based procedure (Dumolin *et al*. 1995) or using the QIAGEN Plant DNeasy Mini Kit (Hilden, Germany). The CTAB protocol included an additional and final treatment of the dissolved DNA with 0.5 µg RNase A at 37 °C for 30 min.

CHLOROPLAST DNA ANALYSIS

A set of conserved primers were used that are reported to amplify chloroplast microsatellite loci in angiosperm species (Weising & Gardner 1999). The primers ccmp 2, 3, 4, 5, 6, 7 and 10 were used (codes, primer sequences and annealing temperatures are described in Weising & Gardner 1999). PCR amplification, gel electrophoresis of PCR products and silver-staining were performed as described by Degen *et al*. (1999). In addition, we carried out a PCR-RFLP analysis of other chloroplast DNA regions. From three published lists of universal primers, five pairs of primers amplifying non-coding cpDNA regions were selected. These were the intergenic spacer regions TF (*trn*T-*trn*F), CS (*psb*C-*trn*S), K1K2 (*trn*K exon1-*trn*K exon2), FV (*trn*F-*trn*V) and VL (*trn*V-*rbc*L). Primer sequences and annealing temperatures are described in Taberlet *et al*. (1991), Demesure *et al*. (1995) and Dumolin-Lapègue *et al*. (1997). In order to detect restriction fragment length and/or restriction site polymorphism, the PCR products were digested (*Taq* I for all regions, plus *Hinf* I for TF, VL and K1K2). PCR amplification, digestion of PCR products, gel electrophoresis and visualization of restriction fragments followed the protocol of Marchelli *et al*. (1998), except that resolution in the PCR-RFLP systems TF–*Hinf* I and VL–*Taq* I was improved by running the gels for 6 and 3.5 h, respectively.

DIG-OXIGENINE LABELLING AND DETECTION OF AFLPS

A non-radioactive DIG-labelled AFLP technology was developed based on radioactive-labelled AFLP technology (Vos *et al*. 1995), and is described in detail in Ziegenhagen *et al*. (2002). The *Eco* RI/*Mse* I system was used as the basic primer–enzyme combination. Primer and adapter sequences were those published in Vos *et al*. (1995). In the final PCR amplification, the *Mse*–site primers carried three additional nucleotides. They are hereafter referred to as the *Mse*–ACT, the *Mse*–TAG and the *Mse*–TTC systems. The resulting gels were documented with X-ray films that were scanned by means of a laser densitometer and the images edited by the software ImagequaNT (Molecular Dynamics, Krefeld, Germany).

DATA SCORING AND ANALYSIS

The cpDNA haplotypes were defined and numbered according to the molecular weight of the largest polymorphic fragment (Fig. 2).

The AFLP gels were scored by eye. An external size standard in the two border lanes of each gel and internal monomorphic bands were used to score the individual banding patterns, and band presence (1) and absence (0) represented in a matrix whose columns corresponded to the individuals or ramets analysed, and rows to the possible bands. Monomorphic and polymorphic bands were included in the matrices. Bands with slight migration differences due to technical anomalies, which are common in gel electrophoresis, were assigned to the same band position.

© 2003 British Ecological Society,

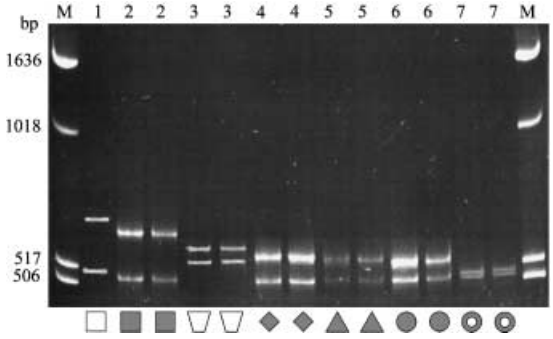

Fig. 2 Seven chloroplast haplotypes of *G. odoratum* as assessed by the PCR-RFLP system TF–*Hinf* I. Except for haplotype TF 1, which was found only once in the Grumsin Forest, two different sprouts or two genets are shown for each haplotype. Symbols (below the gel) correspond to the haplotypes mapped in Figs 1 and 4. Digested PCR products were electrophoretically separated on a 6% non-denaturating polyacrylamide gel, in 1 × Trisborate EDTA buffer at 300 V for 6 h. DNA fragments were stained with ethidium bromide (0.25 μg mL^{-1}) and visualized by UV fluorescence. M = KB Ladder (Gibco BRL Lifetechnologies, Karlsruhe, Germany).

The AFLP patterns obtained at the different scales were transformed into separate 1/0 matrices to analyse any scaling-down effect on the spatial genetic structure. Pairwise similarities were calculated according to the index *s* which is a modification of the Tanimoto Similarity Index, S_T (Deichsel & Trampisch 1985):

$$s = \frac{w + z}{w + x + y + z},$$

where *s* is the similarity index, and *w*, *x*, *y* and *z* are determined from the table of contingency for binary data (Deichsel & Trampisch 1985).

Both joint presence and joint absence of bands are considered to be signs of similarity to allow spatial autocorrelation according to Smouse & Peakall (1999). The similarity index *s* which is the inverse of genetic distance parameters was used to set up the D-matrix (Smouse & Peakall 1999). A geographical distance matrix was calculated from the spatial co-ordinates using Euclidian distances, and used to set up 1/0 matrices for spatial distance classes (X^h-matrix). While Smouse & Peakall (1999) derived and ran permutations on the covariance matrix (C-matrix), we simply permutated the D-matrix. By running 10 000 permutations, confidence intervals of 95% were determined. The confidence intervals under the assumption of random spatial distribution of genotypes and the calculated average similarities within each spatial distance class were plotted in a distogram (Degen & Scholz 1998). Spatial distance classes with less than 10 entries were omitted from the graph, but affected only largest distances.

Two different analyses were performed at the patch level. Inclusion of all samples allowed estimation of average clonal size, whereas, using only distinct genets (all samples having identical genotypes being assumed to be ramets of the same genet) allowed an evaluation of clonal growth vs. local gene flow *via* seed and/or pollen.

Results

DETECTION OF CPDNA POLYMORPHISM

Although all cpDNA microsatellite loci could be amplified by PCR, no length polymorphisms were observed. Only two of the other cpDNA regions analysed (TF and VL) exhibited restriction length polymorphism among the initial 25 test individuals. Two variants could be detected using either the TF–*Hinf* I system or the VL–*Taq* I system, but they covaried so that just two haplotypes were represented by the test individuals (data not shown). System TF–*Hinf* I was later found to be highly polymorphic in the Grumsin Forest whereas only the same two variants in the VL region were detected at the study site (data not shown). We therefore used only TF–*Hinf* I in our spatial analysis. The two haplotypes (TF4 and TF6, see Fig. 2) found in the ancient woodlands were also the most frequent in the Grumsin Forest.

VALIDATION OF AFLPS FOR CLONE IDENTIFICATION

The DIG-labelled AFLP technology was able to discriminate between genets. In the test samples, all four sprouts within each of the two test patches exhibited the same banding patterns with each of the three *Mse*–systems. All systems clearly distinguished between the two patches which are therefore assumed to represent two different genets. Figure 3 illustrates the results for two of the systems (*Mse*–TTC and *Mse*–TAG). DNA extracts from two whorls of the same sprout gave identical AFLP patterns as did ramets within each patch. Similarity indices calculated between two genets from the two patches were nearly the same for each *Mse*–system, although numbers of bands and numbers of polymorphic bands differed (Table 1). The *Mse*–ACT system was chosen for the spatial genetic analysis because it had a high proportion of scorable, polymorphic bands, although all three systems were used in the extensively sampled rectangle.

SPATIAL DISTRIBUTION OF CHLOROPLAST HAPLOTYPES

A total of seven chloroplast haplotypes were found in the Grumsin Forest (Fig. 2). Haplotypes were always reproducible when samples were reanalysed. In order to reliably score haplotypes that differed in length only by a few base pairs, DNA from haplotypes TF 4, TF 5, and TF 6 was run as an internal standard in each gel. Relative frequencies of the predominant haplotypes TF 4 and TF 6 were 0.48 and 0.21. TF 1 occurred only once, TF 7 seems to be clustered in the north-east, and the more common haplotypes (TF 5, 0.12 and TF 2,

© 2003 British Ecological Society,

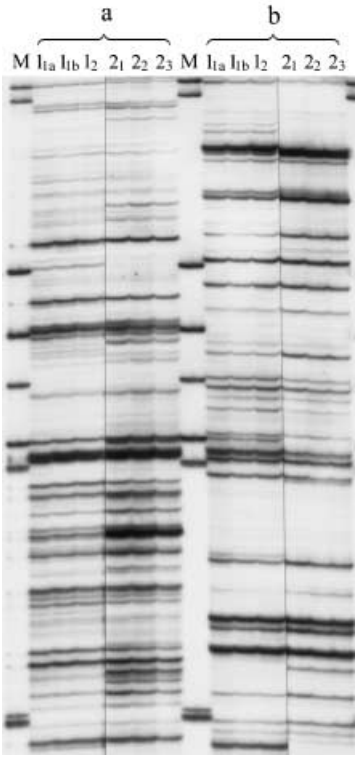

Fig. 3 DIG-labelled AFLP patterns of ramets belonging to two different genets of *G. odoratum* using (a) the *Mse*–TTC system and (b) *Mse*–TAG. 1 and 2 represent samples from the two patches while subscript numbers indicate different ramets within a patch and subscript letters different whorls of the same ramet. M = Size Standard V (Roche Diagnostics, Mannheim, Germany); fragment sizes from the top to the bottom of the gel: 458, 434, 267, 234, 213, 192, 184, 124, and 123 bp.

0.09) were evenly distributed across the study site (Fig. 1).

Four different haplotypes were found in the mid-scale rectangle TF 4 and TF 6 again being the most frequent: TF 3 was only found within this rectangle (Fig. 1). Except in one patch, the three samples exhibited the same haplotypes and adjacent plots (50–60 m away) were sometimes similar. However, across the study site, neighbouring sprouts were often found to represent different haplotypes, and could not therefore be maternal half-sibs.

Table 1 Comparative band statistics between two genets of *Galium odoratum* based on three different selecting primers at the *Mse*–site. *N* = total number of scorable bands; *n* = number of polymorphic bands; and *s* = similarity index

Selecting *Mse*-site primers	*N*	*n*	*s*
(a) *Mse*-ACT system	102	41	0.60
(b) *Mse*-TAG system	96	35	0.64
(c) *Mse*-TTC system	140	56	0.60

Within the sampled patch, only the two dominant haplotypes (TF 4 and TF 6) were detected with TF 4 only occurring in four sprouts on the patch margin (Fig. 4).

CLONE SIZE AND GENET DISTRIBUTION

Identical banding patterns were regarded as indicating that ramets belonged to the same genet, whereas different banding patterns were taken to represent different genets.

Within the mid-scale rectangle, there were 15 genets represented by a single sprout, three by two, and one by three. Three of the multiramet genets were restricted to a single sampling plot, but two ramets of a same genet separated by about 50 m (data not shown). Complete consistency in clonal identification was apparent between the *Mse*-ACT and the *Mse*-TTC systems, but TAG seemed to have less discriminating power (data not shown).

Numerous different genets were found at both scales of spatial resolution in the sampled patch (Fig. 4). The 21 different genets were a mixture of those with numerous and those with single ramets. The ramets of the same genet were mainly found in clusters, varying in size from 1 m up to more than 6 m in diameter (e.g. genet

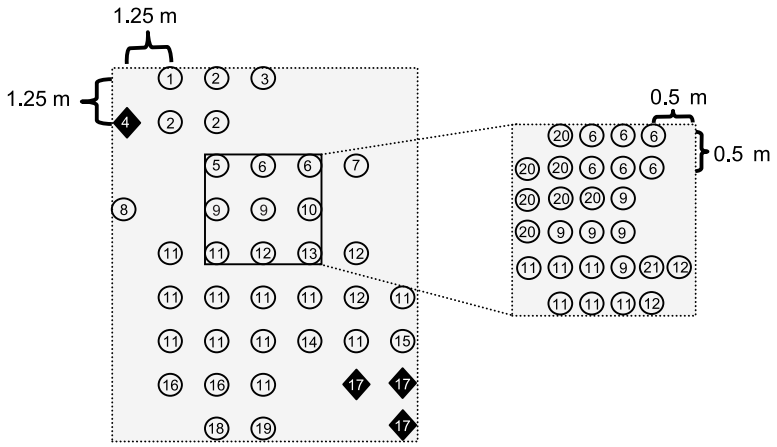

Fig. 4 Schematic drawing of the large sampling patch and distribution of different genets of *G. odoratum*. The entire 5 × 10 m patch was sampled at 1.25 m grid points, and 2.5 × 2.5 m of a densely colonized middle part was sampled at 0.50 m grid points. Numbers represent different genets, circles indicate haplotype TF 6 and diamonds TF 4 (see Figure 2).

© 2003 British Ecological Society,

no. 11, Fig. 4). The densely colonized 'zoom-in' area reflected a similar clonal patchiness to the whole patch.

CLONE SIZE AND DIVERSITY ARE SPATIALLY STRUCTURED

Pairwise similarity indices for genets ranged upwards from 0.46. Within the sampled rectangle, a significant spatial structure was found up to about 100 m, with average similarities significantly higher than expected by chance (Fig. 5), but not at greater distances. The rectangle was therefore colonized by a range of genets, most of which did not exhibit a distinct spatial genetic structure.

At the patch level there was spatial genetic structure at small scales (Fig. 6). When all ramets were included in the analysis, a strong spatial structure was detected at both scales (Fig. 6a,c). In general, average similarities decreased with increasing distance, and within the first two distance classes, were higher than expected by chance. A further analysis based on just one ramet per genet showed decreased similarities (Fig. 6b,d). Although the spatial structure was maintained at the

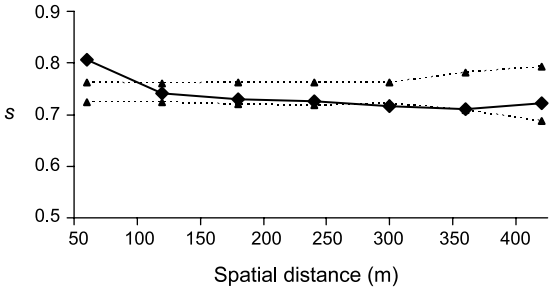

Fig. 5 Distogram of average similarities (s) obtained from AFLP patterns for *G. odoratum* within the 10×400 m long rectangle. The 95% confidence interval of 10 000 permutations is marked by dotted lines.

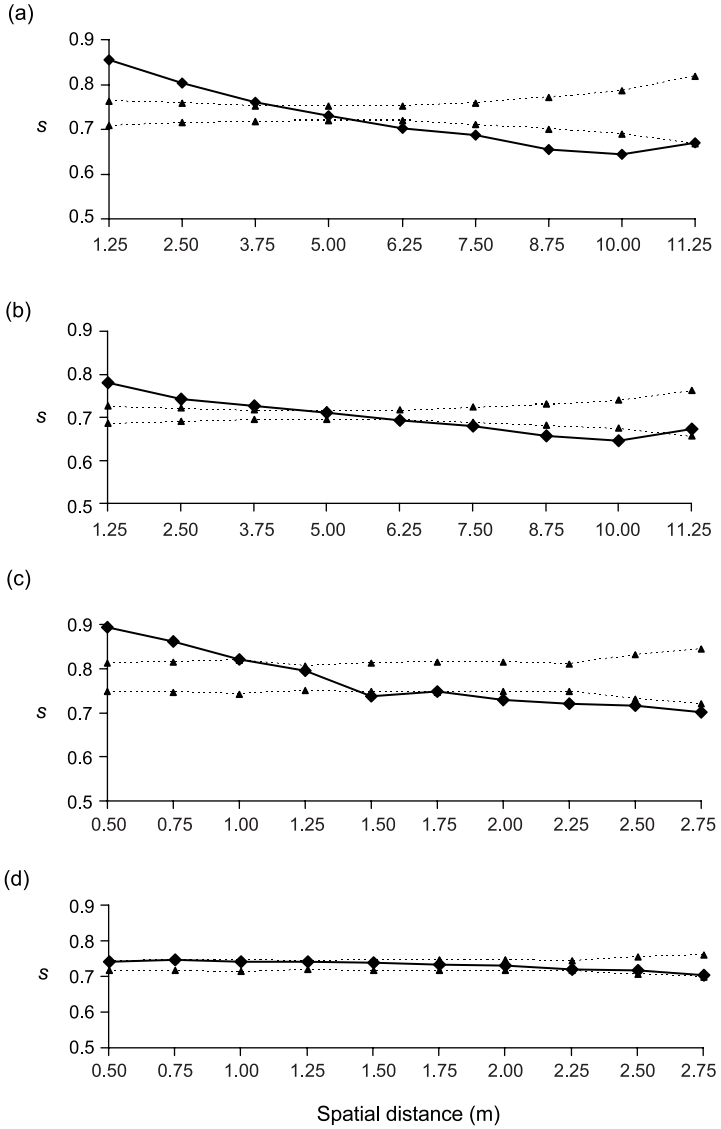

Fig. 6 Distograms of average similarities (s) obtained from AFLP patterns for *G. odoratum* at the patch level. The 95% confidence intervals of 10 000 permutations are marked by dotted lines. (a) and (b) cover the whole patch (5×10 m), and (c) and (d) only the central area (2.5×2.5 m). (a) and (c) include all samples, and (b) and (d) only one ramet per genet.

© 2003 British Ecological Society,

whole-patch level (Fig. 6b), indicating a higher relatedness of neighbours, there was no longer any significant spatial genetic structure in the 'zoom-in' patch (Fig. 6d), indicating that any structure here was mainly due clonal growth.

Discussion

Recent research on indicator species of ancient woodlands has focused on their dispersal abilities (Brunet & von Oheimb 1998; Heinken 2000). We developed and adapted neutral DNA markers to investigate the efficiency of dispersal of *Galium odoratum*, a species with both generative and vegetative propagation, in a long established woodland.

NEW MARKERS FOR SEED DISPERSAL AND CLONE IDENTIFICATION IN *G. ODORATUM*

A cpDNA marker was used to trace maternal lineages and AFLPs to analyse clonal patterns. Maternal inheritance is the rule for chloroplasts of angiosperms (Sears 1980), and *G. odoratum* does not belong to the genera and families that are known exceptions (Hagemann & Schröder 1989). We observed no individuals with two different TF haplotypes confirming the absence of biparental inheritance. The degree and distribution of cpDNA variation could therefore be used to infer patterns of seed dispersal within the study area.

For clone identification, it was possible to distinguish reliably between genets using a single AFLP primer–enzyme combination, as in other species. Members of an oak half-sib family could be distinguished from each other by means of a single primer–enzyme combination (Ziegenhagen *et al*. 1999; Ziegenhagen *et al*. 2002). The biparentally inherited AFLP markers were complementary to the maternally inherited cpDNA marker, thus allowing estimation of gene flow *via* pollen.

SMALL-SCALE GENETIC DIVERSITY: INFERENCES ABOUT SEEDLING RECRUITMENT AND GENE FLOW

G. odoratum exhibited clonal diversity on a small scale, with the patches in which it typically occurs containing numerous different genets, even using a 0.5×0.5 m grid. There are similar reports of small-scale clonal diversity of other clonal woodland species, e.g. for *Anemone nemorosa* L. (Holderegger *et al*. 1998; Stehlik & Holderegger 2000), *Uvularia perfoliata* L. (Kudoh *et al*. 1999) and *Viola riviniana* RCHB. (Auge *et al*. 2001). Genets tended to form small (0–6 m diameter) clusters of ramets and did not intermingle. Two ramets of the same genet were, however, found about 50 m apart in the mid-scale rectangle, probably due to fragmentation followed by dispersal. Similar findings have been reported in other clonal species such as *Vaccinium stamineum* L. (Kreher *et al*. 2000). In *A. nemorosa*, a clone with over six times the average clonal diameter (Stehlik & Holderegger 2000) might have been fragmented and dispersed by foraging wild boar (Proznikow 1994).

We conclude, that in general migration, and/or colonization, take place by repeated seedling recruitment (Eriksson 1993). Small-scale diversity in maternal lineages, indicates that neighbours often originated from different seed mothers. The occurrence of less frequent haplotypes in a neighbourhood of a frequent haplotype suggests that there may also be occasional non-local seed dispersal events. Leptocurtic dispersal is common in postglacial colonization processes (Nichols & Hewitt 1994; Ibrahim *et al*. 1996; Petit *et al*. 2001). Although, we infer a combination of local events and dispersal in excess of the spatial scale analysed, we cannot explicitly term this 'leptokurtic' because we know neither the distance nor the frequency of non-local events.

The spatial autocorrelation analysis provided additional evidence for leptokurtic-like gene dispersal in *G. odoratum*. The genetic structure in the centre of the analysed patch was shaped by vegetative propagation and by non-local rather than small-scale local gene flow. Outcrossing with non-local pollen is feasible for this insect-pollinated species (Jäger & Werner 2002). Most of the individuals in the patch carried the same cpDNA haplotype suggesting repeated seed recruitment of maternal half-sibs as reported for *A. nemorosa* patches (Stehlik & Holderegger 2000).

THE ROLE OF DISPERSAL MECHANISMS FOR COLONIZING NEW HABITATS

Our results suggest recruitment from seeds of both local and non-local origin. *Galium odoratum* individuals do occasionally occur well away from the migration front line (Brunet & von Oheimb 1998), and seed morphology suggests that mammals with large ranges could be responsible for this dispersal. However, only 11 of 266 diaspores recovered from the coats of wild boars and 1 of 3702 from dog fur belonged to *G. odoratum* (Luftensteiner 1982; Heinken 2000). This may indicate an extremely low frequency of non-local seed dispersal or assumptions about the main vectors may be incorrect. Very low frequencies (1×10^{-7}) can enhance colonization speed in oaks (Le Corre *et al*. 1997). *Galium odoratum* clones originating from dispersal events which happened long ago may be growing in close proximity to younger clones. Woodland herbs are long-lived, e.g. *Hepatica nobilis* MILL. and *Sanicula europaea* L. may have maximum ages of about 360 and 185 years, respectively (Inghe & Tamm 1985). Although Brunet *et al*. (2000) claimed that it is the spatial distance between source populations (ancient woodland) and recent woodlands alone which determines the species composition of the recent woodlands our results neither confirm nor reject this hypothesis. Are ecological factors also likely to prevent colonization into recent woodlands by *G. odoratum*? The species' ability to

© 2003 British Ecological Society,

colonize habitats adjacent to ancient woodlands does not correlate with habitat heterogeneity (Brunet & von Oheimb 1998; Honnay *et al*. 1999), although it is enhanced on rich soils (Dzwonko & Gawronski 1994) and delayed by strong competition with grass and *Urtica dioica* (Hermy *et al*. 1993). Clones of another *Galium* species, *G. boreale*, did correlate with habitat heterogeneity as they showed different tolerances towards low soil pH (Dale & Elkington 1984).

If species-specific dispersal mechanisms are a constraint to colonization, spatial genetic structure would differ in woodlands with less spatio-temporal continuity. We are therefore investigating ancient and recent woodlands separated by a range of distances as well as aiming at simulation studies to model gene flow.

Acknowledgements

We would like to give our thanks to Sigune Weinert for DNA extraction, and to Simone Meier, Gisela Rühl and Gisela Thomas for the field work (all ZALF e.V., Müncheberg, Germany). We gratefully appreciate the invitation made to M.W. to participate in a working group of the National Center for Ecological Analysis and Synthesis [NCEAS, a Center funded by NSF (Grant no. DEB-94–21535)], University of California at Santa Barbara, USA (1999). During this workshop, M.W. discussed at length with Victoria L. Sork and Peter Smouse the topics of ancient woodland indicator species and how to analyse them. Furthermore, we are grateful to Rémy J. Petit (INRA Bordeaux, France) for numerous fruitful discussions. Sarah Rendell (Oxford Forestry Institute, UK) kindly gave linguistic advice on the manuscript. We appreciate highly the constructive criticism by anonymous referees.

References

Auge, H., Neuffer, B., Erlinghagen, F., Gruppe, R. & Brandl, R. (2001) Demographic and random amplified polymorphic DNA analyses reveal high levels of genetic diversity in a clonal violet. *Molecular Ecology*, **10**, 1811–1819.

Brunet, J. (1994) Der Einfluß von Waldnutzung und Waldgeschichte auf die Vegetation südschwedischer Laubwälder. *Norddeutsche Naturschutzakademie Berichte*, **7**, 96–101.

Brunet, J., Oheimb, G. & Diekmann, M. (2000) Factors influencing vegetation gradients across ancient-recent woodlands borderlines in southern Sweden. *Journal of Vegetation Science*, **11**, 515–524.

Brunet, J. & von Oheimb, G. (1998) Migration of vascular plants to secondary woodlands in southern Sweden. *Journal of Ecology*, **86**, 429–438.

Dale, A. & Elkington, T.T. (1984) Distribution of clones of *Galium boreale* L. on Widdybank Fell, Teesdale. *New Phytologist*, **96**, 317–330.

Degen, B. & Scholz, F. (1998) Spatial genetic differentiation among populations of European beech (*Fagus sylvatica* L.) in western Germany as identified by geostatistical analysis. *Forest Genetics*, **5**, 191–199.

Degen, B., Streiff, R. & Ziegenhagen, B. (1999) Comparative study of genetic variation and differentiation of two pedunculate oak (*Quercus robur*) stands using microsatellite and allozyme loci. *Heredity*, **83**, 597–603.

Deichsel, G. & Trampisch, H.J. (1985) *Clusteranalyse und Diskriminanzanalyse*. Gustav Fischer Verlag, Stuttgart.

Demesure, B., Sodzi, N. & Petit, R.J. (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, **4**, 129–131.

Dumolin, S., Demesure, B. & Petit, R.J. (1995) Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theoretical and Applied Genetics*, **91**, 1253–1256.

Dumolin-Lapègue, S., Pemonge, M.H. & Petit, R.J. (1997) An enlarged set of consensus primers for the study of organelle DNA in plants. *Molecular Ecology*, **6**, 393–397.

Dzwonko, Z. & Gawronski, S. (1994) The role of woodland fragments, soil types, and dominant species in secondary succession on the western Carpathian foothills. *Vegetatio*, **111**, 149–160.

Ellenberg, H., Weber, H., Düll, R., Wirth, V., Werner, W. & Paulißen, D. (1992) Zeigerwerte Von Pflanzen in Mitteleuropa, 2nd edn. *Scripta Geobotanica*, **18**, 1–258.

Eriksson, O. (1993) Dynamics of genets in clonal plants. *Trends in Ecology and Evolution*, **8**, 313–316.

Fischer, A. (1987) Untersuchungen zur Populationsdynamik am Beginn von Sekundärsukzessionen. *Dissertationes Botanicae*, **110**.

Hagemann, R. & Schröder, M.-B. (1989) The cytological basis of the plastid inheritance in angiosperms. *Protoplasma*, **152**, 57–64.

Heinken, T. (2000) Dispersal of plants by a dog in a deciduous forest. *Botanisches Jahrbuch für Systematik*, **122**, 449–467.

Hermy, M., van den Bremt, P. & Tack, G. (1993) Effects of site history and woodland vegetation. *European Forest Reserves* (eds M.E.A. Broekmeyer, W. Vos & H. Koop), pp. 219–232. Pudoc Scientific Publishers, Wageningen.

Holderegger, R., Stehlik, I. & Schneller, J.J. (1998) Estimation of the relative importance of sexual and vegetative reproduction in the clonal woodland herb *Anemone nemorosa*. *Oecologia*, **177**, 105–107.

Honnay, O., Hermy, M. & Coppin, P. (1999) Effects of area, age and diversity of forest patches in Belgium on plant species richness, and implications for conservation and reforestation. *Biological Conservation*, **87**, 73–84.

Ibrahim, K., Nichols, R.A. & Hewitt, G.M. (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282–291.

Inghe, O. & Tamm, C.O. (1985) Survival and flowering of perennial herbs. IV. The behaviour of *Hepatica nobilis* and *Sanicula europaea* on permanent plots during 1943–81. *Oikos*, **45**, 400–420.

Jäger, E.J. & Werner, K., eds. (2002) *Exkursionsflora Von Deutschland*. Bd. 2. Gefäßpflanzen Spektrum Akademischer Verlag, Stuttgart.

Kreher, S.A., Foré, S.A. & Collins, B.S. (2000) Genetic variation within and among patches of the clonal species, *Vaccinium stamineum* L. *Molecular Ecology*, **9**, 1247–1252.

Kudoh, H., Shibaike, H., Takasu, H., Whigham, D.F. & Kawando, S. (1999) Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. *Journal of Ecology*, **87**, 244–257.

Le Corre, V., Machon, N., Petit, R.J. & Kremer, A. (1997) Colonization with long-distance seed dispersal and genetic structure of maternally inherited genes in forest trees: a simulation study. *Genetic Research*, **69**, 117–125.

Lippert-Hambacher, E. (1992) *Untersuchungen zur Verbreitungsbiologie ausgewählter Waldbodenpflanzen im Rekultivierungsgebiet Sophienhöhe* (Tagebau Hambach, Rheinisches Braunkohlenrevier). PhD Thesis, University of Cologne, Germany.

Luftensteiner, H.W. (1982) Untersuchungen zur Verbreitungsbiologie von Pflanzengemeinschaften an vier Standorten in Niederösterreich. *Bibliotheca Botanica*, **135**.

© 2003 British Ecological Society,

Marchelli, P., Gallo, L., Scholz, F. & Ziegenhagen, B. (1998) Chloroplast DNA markers revealed a geographical divide across Argentinean southern beech *Nothofagus nervosa* (Phil.) Dim. et Mil. distribution area. *Theoretical and Applied Genetics*, **97**, 642–646.

Matlack, G.R. (1994) Plant species migration in a mixed-history forest landscape in eastern North America. *Ecology*, **75**, 1491–1502.

Nichols, R.A. & Hewitt, G.M. (1994) The genetic consequences of long distances dispersal during colonization. *Heredity*, **72**, 312–317.

Peterken, G.F. (1981) *Woodland Conservation and Management*. Chapman & Hall, London & New York.

Petersen, P.M. & Philipp, M. (2001) Implantation of forest plants in a wood on former arable land: a ten year experiment. *Flora*, **196**, 286–291.

Petit, R.J., Bialozyt, R., Brewer, S., Cheddadi, R. & Comps, B. (2001) From spatial patterns of genetic diversity to postglacial migration processes in forest trees. *Plants Stand Still But Their Genes Don't* (ed. J. Silvertom), pp. 295–318. Blackwell Science Publications, Oxford.

Proznikow, E. (1994) Demography of *Anemone nemorosa* L. in dry-site deciduous forest (Tilio-Carpinetum) in the Bialowieza Forest. *Ekologia Polska*, **42**, 155–172.

Rackham, O. (1980) *Ancient Woodlands in the British Landscape*. Edward Arnold, London.

Sears, B.B. (1980) The elimination of plastids during spermatogenesis and fertilization in the plant kingdom. *Plasmid*, **4**, 233–255.

Smouse, P.E. & Peakall, R. (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.

Stehlik, I. & Holderegger, R. (2000) Spatial genetic structure and clonal diversity of *Anemone nemorosa* in late successional deciduous woodlands of Central Europe. *Journal of Ecology*, **88**, 424–435.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, **17**, 1105–1109.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Mornes, M., Fritjers, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Weising, K. & Gardner, R.C. (1999) A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. *Genome*, **42**, 9–19.

Wulf, M. (1994) Überblick zur Bedeutung des Alters von Lebensgemeinschaften, dargestellt am Beispiel 'historisch alter Wälder'. *Norddeutsche Naturschutzakademie Berichte*, **7**, 3–14.

Wulf, M. (1997) Plant species as indicators of ancient woodland in northwestern Germany. *Journal of Vegetation Science*, **8**, 635–642.

Ziegenhagen, B., Brettschneider, R., Kuhlenkamp, V. & Fladung, M. (2002) Non-radioactive DIG-labelled AFLPs for application in forest trees. *Luminescence Biotechnology: Instruments and Applications* (ed. K. van Dyke), pp. 211–222. CRC Press, Boca Raton.

Ziegenhagen, B., Kuhlenkamp, V., Brettschneider, R., Scholz, F., Stephan, B.R. & Degen, B. (1999) DIG-labelled AFLPs in oaks – A DNA marker for reconstruction of full- or half-sib family relationships? *Which Marker for Which Purpose?* Final compendium of the research project 'Development, optimization and validation of molecular tools for assessment of biodiversity in forest trees' in the European Union DGXII Biotechnology FW IV Research Programme *Molecular Tools for Biodiversity* (ed. E.M. Gillet). URL, *http://www.sub.gwdg.de/ebook/y/1999/whichmarker/index.htm.*

Received 11 June 2002
revision accepted 1 April 2003

© 2003 British Ecological Society,