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ORIGINAL PAPER

Molecular data indicate multiple independent colonizations of former lignite mining areas in Eastern Germany by *Epipactis palustris* (Orchidaceae)

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Abstract Former lignite mining areas in Eastern Germany are valuable secondary habitats for many plant and animal species endangered in the natural landscape. Here, we present a study on genetic structure and diversity of 16 populations of the threatened orchid *Epipactis palustris* (Orchidaceae) from five mining pits and 11 natural habitats, which we carried out in order to ascertain how many times this species immigrated into former lignite mining areas, and where the source populations are located. We used two different anonymous genetic marker methods, random amplified polymorphic DNA (RAPD) and microRNA-primed genomic fingerprinting (miRPF) to analyze patterns of genetic variation. Results of a multivariate analysis based on asymmetric Soerensen similarity, principal coordinate analysis and a neighbor-joining cluster analysis indicate high within population-variability and a moderate genetic differentiation among *E. palustris* populations. We found no differences between genetic diversity values of populations from former mining areas and those of natural habitats. Thus, we could not find evidences for genetic bottlenecks in the mining populations due to founder events. Source populations are predominantly close surrounding populations as geographic distance and genetic dissimilarity were correlated. However, exchanges may reach beyond 125 km and repeated independent colonization events are highly likely.

Keywords Colonization · Donor population · *Epipactis palustris* · Genetic diversity · Immigration · Mining pits · miRPF · Population structure · RAPD

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Introduction

Surface mining sites of several types (e.g. sand, stone, limestone, clay, minerals, and ores) provide for several decades now the unique opportunity to analyze recolonization and spontaneous or initiated restoration of largely destroyed and significantly altered landscapes (e.g. Prach 1987; Twigg et al. 1989; Wiegleb and Felinks 2001). These studies have offered insights into the development of species communities, the ability of plants and animals to migrate and to bridge unsuitable habitats or the effects of competition as well as different stress factors (e.g. Antonovics and Bradshaw 1970; Prach and Pyšek 1999; Brandle et al. 2000; Mrzljak and Wiegleb 2000; Martinez-Ruiz and Marrs 2007). These studies also facilitate developing models to predict and thus possibly manage recolonization and restoration processes in order to support and protect endangered species and communities (e.g. Bradshaw 1997; Choi 2004; Csecserits et al. 2007; del Moral 2007).

Extraction by surface mining destroys entire landscapes and the interlinked ecosystems (e.g. Bradshaw 2000). Therefore, it is important to examine how impact of mining can be mitigated and what restoration strategy may achieve the best possible result. Various studies showed that large-scale areas reserved for spontaneous succession can enhance biodiversity on former mineral mining (e.g. Prach and Pyšek 2001; Prach 2003; Morris et al. 2006; Rehounková and Prach 2006). In mined sites that are not restored, a high heterogeneity in abiotic conditions such as geomorphology, hydrology as well as nutrient-deficiency resulted in a large number of niches for recolonization of species adapted to open habitats (e.g. Bradshaw 1983; Ash et al. 1994; Schulz and Wiegleb 2000). In relatively well-studied mining sites of Saxony-Anhalt, which were up to 60 years old, 14% of all rare and threatened plant species of Saxony-Anhalt could be observed (Tischew and Kirmer 2007), among them 16 orchid species. Kirmer et al. (accepted); see also Tischew and Kirmer (2003) showed that these species can bridge distances of at least 17 km in colonization processes. The poorly structured agricultural landscape in the surroundings of the mining sites along with specific thermal conditions at source sites support the influx of seeds and diaspores by wind into the mining pits (Klotz et al. 2000; Tischew and Kirmer 2003; Kirmer et al. accepted). Ash et al. (1994) as well as Bradshaw (1983) likewise observed that species with very small seeds and specialized soil preferences (e.g. orchids) bridging distances up to 40 km to reach suitable sites (e.g. industrial waste heaps).

Nutrient-deficient but calcium carbonate-rich fens and swamps with an impeded colonization by shrubs and trees offer long-term habitats for orchids in mining areas. *Epipactis palustris* (L.) Crantz, one of the endangered orchids in Saxony-Anhalt disappeared on 90% of its natural habitats due to effects of eutrophication and land use changes. In contrast are records of *E. palustris* occurrences on an increasing number of mined sites in the southern part of Saxony-Anhalt (19 sites in 2004). In the south-eastern part of Saxony-Anhalt the species colonizes nearly exclusively former lignite mining areas (FLMA; Baasch and Seppelt 2004). On at least five of these sites population size exceeds several thousands of ramets (Hardtke and Ihl 2000; Baasch and Seppelt 2004). According to these findings we assume that mined areas function both as sinks and sources for colonization processes.

Molecular markers have been shown to be valuable tools to analyze migration and founder events (Barrett and Shore 1989; McCauley et al. 1995; Tremetsberger et al. 2003; Pleines and Blattner 2008). Information on migration routes as well as on potential source populations, and the amount of genetic diversity occurring in a certain landscape are of great value to analyze gene flow, dispersal and colonization, and to develop conservation strategies (Durka et al. 2005). However, apart from Mengoni et al. (2000, 2001), Krüger

et al. (2002), Brock et al. (2007) and Reisch (2007) comprehensive genetic studies of plant populations in primary versus secondary habitats are still missing.

We analyzed patterns of microRNA-primed fingerprinting (miRPF; Blattner et al. submitted) and random amplified polymorphic DNA variation (RAPD; Welsh and McClelland 1990; Williams et al. 1990) from individuals of 16 populations of *E. palustris* (11 from natural and five from secondary habitats). RAPDs were often criticized because the short primers (10 nucleotides length) used in PCR resulted sometimes in low reproducibility of the fragment patterns among different laboratories. Although generally similar to the RAPD procedure, miRPF overcomes this restriction by utilizing a group of conserved microRNA genes and target sites (18–20 nucleotides length) as primer binding sites. miRPF work well on the population level, where they are able to discern clones and quite similar individuals and provide insight in population structure.

With the present study we addressed and assessed the following questions: (1) Did *E. palustris* populations in former mining pits originate due to single or due to multiple colonization events?. (2) Where are possible source populations located and can we identify these using anonymous molecular markers?. (3) Is the genetic diversity of FLMA populations reduced in comparison to natural populations due to founder events?.

Materials and methods

Species, study region and plant sampling

Epipactis palustris is a perennial summer-green orchid of Central Asia and Europe and inhabits lowlands and habitats up to 2000 m a.s.l (Baum 1998). It colonizes high humidity habitats like fens, lakesides, reeds or meadows but is also found in secondary habitats such as water logging sites in mining areas (Oberdorfer 1994; Rothmaler 1996; Baasch and Seppelt 2004) and is currently classified as vulnerable (Welk 2002). *E. palustris* blossoms between May and August and is pollinated by hymenoptera, diptera or beetles (Baum 1998). The species is self-compatible (Ziegenspeck 1936), and autogamy is possible but rare in nature (Nilsson 1978; Sebald et al. 1998). The capsules contain thousands of dust-like seeds, which lack any endosperm resulting in a very low thermal velocity of about 0.2 m s^{-1} and a high wind dispersal potential (Tackenberg 2001; Tackenberg et al. 2003). Therefore, seeds can bridge large distances particularly when supported by thermal updrafts in open landscapes. Haas (2000) assumes that after establishment of the species clonal growth may result in a fast population expansion.

Our study sites are located in eastern Germany (Fig. 1). Five lignite mining areas were chosen to study *E. palustris* populations: Domsen (Dom), Jaucha (Jau) and Pirkau (Pir), which are part of the Zeitz–Weißenfels–Hohenmölsen coal district and Kayna (Kay) and Roßbach (Ross) situated in the Geiseltal coal district. Mining ended at least 25 years ago and primary succession is in progress. We analyzed seven natural populations along the main wind direction to the FLMAs in northern Thuringia and Saxony-Anhalt as potential donor populations. These populations were clearly smaller in size than the secondary populations in the mining pits. We found these *E. palustris* populations in small sedge reeds or *Molinia* meadows often surrounded by agricultural lands. Additionally, our study included four natural *E. palustris* populations from Baden-Württemberg, Bavaria, Thuringia and Mecklenburg-Western Pomerania, which were included as outgroup populations. A population was defined as a group of plants separated from their closest conspecific by at least 4 km. The distances between two populations were between 4 and

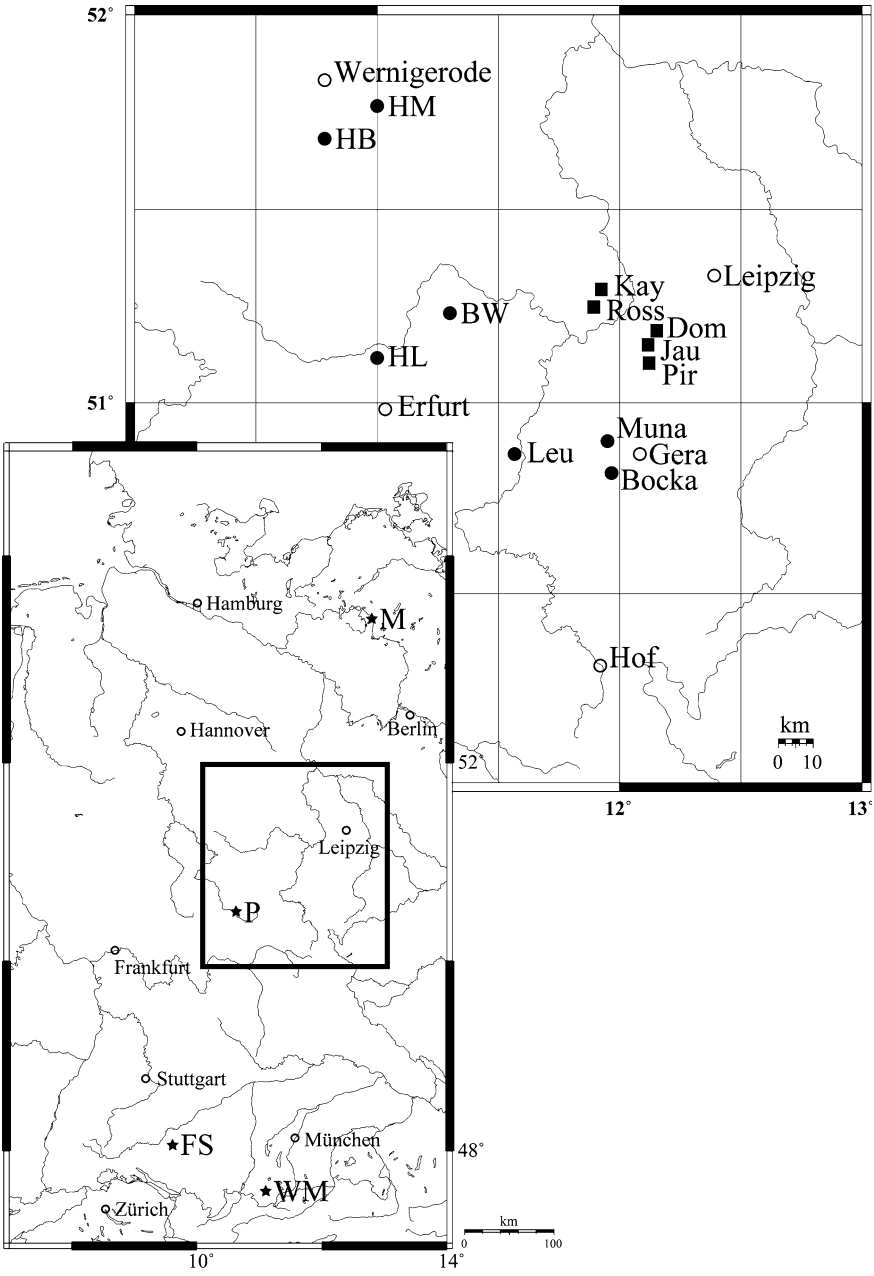

Fig. 1 Locations of the sampled *E. palustris* populations (FLMA: square, surrounding populations: dots, outgroups: asteroids). Populations are abbreviated as indicated in Table 1

660 km and populations within 125 km were referred to as surrounding populations. Population sizes varied from 150–1,500 individuals in natural populations to 5,000–1,000,000 individuals in the FLMAs. The molecular analysis included DNA from 3 to 15

Table 1 Populations, sample sizes, percentage of polymorphic loci, mean Soerensen dissimilarity among plants from a given population, and genetic diversity of the study populations of *E. palustris*

Population	Sample size	% Polymorphic bands	Average Soerensen dissimilarity	Average genetic diversity
FLMA				
Domsen (Dom)	21 (8)	67.3 (55.3)	0.24 (0.25)	0.20 (0.24)
Jaucha (Jau)	15 (8)	69.7 (55.2)	0.22 (0.22)	0.24 (0.22)
Kayna (Kay)	23 (8)	77.8 (45.4)	0.22 (0.18)	0.21 (0.18)
Pirkau (Pir)	18 (8)	73.2 (60.2)	0.28 (0.30)	0.23 (0.26)
Rossbach (Ross)	24 (8)	75.6 (60.5)	0.27 (0.26)	0.25 (0.25)
Surroundings				
Bocka	7	50.6	0.23	0.21
Burgwenden (BW)	7	41.1	0.17	0.16
Harzer Bachtäler (HB)	13 (8)	68.5 (51.2)	0.24 (0.23)	0.24 (0.21)
Haßleben (HL)	8	48.4	0.24	0.19
Hammelwiese (HM)	7	43.0	0.20	0.18
Leutra (Leu)	13 (8)	63.8 (47.1)	0.22 (0.21)	0.20 (0.18)
Muna	7	57.5	0.27 (0.27)	0.23
Outgroups				
Federsee (FS)	3	38.1	0.43	0.25
Pferdehut (P)	4	40.2	0.45	0.26
Müritz (M)	5	41.7	0.29	0.20
Weidmoos (WM)	15 (8)	50.0 (43.8)	0.20 (0.19)	0.14 (0.16)

Figures in brackets refer to the reduced sample sizes

plants per population for outgroups, 7 to 13 from surrounding primary populations, and 15 to 24 from FLMAs (Table 1). The samples were collected randomly in June 2003. We sampled from 0.5 to 100 m distance between sample sites along transects to detect potential clonal growth.

Genetic analyses

DNA was extracted in two different ways. Most samples were extracted from 10 to 25 mg silica-gel dried leaf material with the Qiagen MagAttract Plant DNA extraction kit, while for some samples the Qiagen DNeasy Plant Mini Kit was used following the standard protocols of the manufacturer (Qiagen). Extracted DNA was checked on 1.0% agarose gels stained with ethidium bromide and diluted to equal amounts of DNA.

For the miRPF analysis we used reaction volumes of 10 µl containing 5–10 ng of total DNA, 0.1 µM of the respective miRPF primer (Table 2), 0.2 mM of each dNTP, 0.1 U Taq (Qiagen), in the reaction buffer provided by the manufacturer (including 1× Q-Solution to reduce amplification problems due to secondary structures and a final concentration of 3 mM $MgCl_2$). PCR was carried out in a GeneAmp PCR System 9700 Thermal Cycler (PE Biosystems) or in an Eppendorf Mastercycler gradient as followed. An initial denaturation at 94°C for 3 min was followed by 10 cycles of a touch-down protocol with denaturation at 94°C for 30 s, annealing temperatures (decreasing by 0.5°C each cycle) at 56.0–51.5°C for 1 min and elongation at 70°C for 2 min. This touch-down part was followed by 30 cycles

Table 2 Primer used in the analysis, PCR parameters, and number of analyzed bands per primer

Primer	Sequence (5′–3′)	Annealing temperature (°C)	Elongation time (min)	Number of analyzed bands
MiRPF				
miRPF-632	TGCCTGGCTCCCTGTATGCC	56–51	2	12
miRPF-632r	TGGCATACAGGGAGCCAGGC	56–51	2	10
miRPF-636r	CTGGATGCAGAGGYTTATCG	50–45	3:30	8
miRPF-644	TCGGACCAGGCTTCATTCCC	56–51	2	16
miRPF-644r	GGGGAATGAAGCCTGGTCCG	56–51	2	13
miRPF-650	TGAAGCTGCCAGCATGATCT	54–49	2	15
miRPF-655	TGATTGAGCCGYGYCAATAT	52–47	3:30	15
				∑89 Ø 12
RAPD				
C14	TCCGCTCTGG	43–37	2	10
D06	ACCTGAACGG	43–37	2	9
G02	GGCACTGAGG	43–37	2	7
G13	CTCTCCGCCA	43–37	2	7
				∑33 Ø 8

of denaturation at 94°C for 30 s, annealing at 51°C for 1 min and elongation at 70°C for 2 min with a final elongation step at 70°C for 10 min. Annealing temperature and elongation time can vary for different primers (Table 2). DNA fragments were separated on a 1.6% agarose gel stained with ethidium bromide by electrophoresis with a Tris-borate-EDTA (TBE) buffer system at 120 V for 4 h. For the RAPD analysis DNA was amplified in reaction volumes of 10 µl containing 5–10 ng/µl of total DNA, 0.5 µM of the respective RAPD primer (Table 2), 0.2 mM of each dNTP, 0.1 U Taq (Qiagen), in the reaction buffer provided by the manufacturer (including 1× Q-Solution to reduce amplification problems due to secondary structures and a final concentration of 2.5 mM $MgCl_2$). PCR was carried out in the GeneAmp PCR System 9700 Thermal Cycler (PE Biosystems) or in the Eppendorf Mastercycler gradient. Initial denaturation took place at 94°C for 3 min followed by 10 touch-down cycles with denaturation at 94°C for 30 s, annealing (temperatures decreasing by 0.5°C each cycle) at 43–38.5°C for 1 min and elongation at 72°C for 2 min. After the touch-down part 32 cycles of denaturation at 94°C for 30 s, annealing at 37°C for 1 min and elongation at 72°C for 2 min with a final cycle at 72°C for 10 min before cooling were conducted. Separation took place by electrophoresis on a 1.6% ethidium bromide-stained agarose gel at 120 V for 4 h. All analyses were repeated twice and only reproducible bands were scored. Photographs of the gels were taken and fragment scoring was done on enlarged prints of the gel photos.

Statistical analyses

The miRPF and RAPD fragments were scored as a binary matrix where 1 was presence and 0 absence for all clearly detectable and reproducible bands was used. We combined the results of the miRPF and RAPD analyses in a single data matrix, which was used for all

following steps. The analysis primarily based on a multivariate, band-based approach (Bonin et al. 2007). We used Soerensen similarity (equal to the coefficient of Nei and Li 1979), which is an asymmetrical index emphasising on bands shared among individuals. Values were transformed to a distance/dissimilarity measure by subtracting them from 1. A principal coordinate analysis (PCoA) on square-root transformed dissimilarities was performed to illustrate the general patterns among populations. Additionally, we tested differences between populations and the relationship between geographic distance (raw and log-transformed) and mean genetic distance with Mantel Tests (Mantel 1967) based on Soerensen dissimilarities. Dissimilarities were also tested against a design matrix, where samples originating from the same populations were assigned a dissimilarity of '0', and samples from different populations a value of '1'. The Mantel tests were run using 9,999 permutations to assess significance. We performed the analyses with PC-ORD 3.15 (McCune and Mefford 1997) and CANOCO 4.5 (ter Braak and Smilauer 2002). In a second approach, we used the software PAUP* 4.0b10 (Swofford 2002) to calculate a neighbor-joining tree (NJ) using the Nei-Li distance, and Arlequin (Version 2.000; Schneider et al. 2000) to examine the genetic diversity of populations (percentage of polymorphic loci and average gene diversity over loci among all members of populations). Additionally we calculated Soerensen dissimilarity among plants because application of standard measures of genetic diversity using dominant markers relies on several assumptions including presence of Hardy–Weinberg equilibrium. The dissimilarity matrix was then used to calculate mean dissimilarity among individuals, as well as within and among populations.

In the FLMA populations, we collected and analyzed more specimens than in the natural populations. Thus, to minimize the influence of the sample size in our study populations, 8 samples were randomly chosen from the larger data set for analysis at the population level (reduced data set; Hurlbert 1971; Jakob et al. 2007; see Table 1). Except for the reduced number of polymorphic loci (*t*-test, $P = 0.054$), no statistic was affected by reducing sample size (Table 1), confirming that our molecular assessments are relatively insensitive to sampling intensity (for RAPD see Nybom and Bartish 2000).

Results

The 11 primers used in the miRPF and RAPD analyses of 190 individuals from 16 populations of *E. palustris* yielded a total of 122 amplified and analyzed DNA bands of which 120 were polymorphic (98.4%). MiRNA-primed fingerprinting primers produced 89 informative bands, RAPDs 33. Fragment sizes ranged from about 200 to 2,200 base pairs. Each plant was distinguishable as a single marker phenotype. The total number of bands per primer varied from 16 (miRPF-644) to 7 (RAPD G13); the percentage of polymorphic bands per primer from 38 to 78% (Table 2).

The PCoA indicated no simple genetic pattern and showed no clear separation, neither between populations nor between regions or groups. However, whereas the populations of FLMAs and surroundings strongly overlap, outgroups occupy mainly the left hand section of the ordination diagram. Still, samples from the surrounding populations occasionally occurred among those from the FLMAs. In concordance, genetic differentiation among populations was weak (standardized Mantel-correlation; r_M full data set $= 0.151$, r_M reduced data set $= 0.147$) but nonetheless highly significant ($P < 0.0001$ in both cases). The relationship between genetic similarity among populations and geographic distance was negative but also weak: the matrix of 120 mean Soerensen similarities among all 16

populations only marginally correlated with the corresponding matrix of geographic distances (Mantel test: r_M full data set $= -0.356$, $P = 0.070$; r_M reduced data set $= -0.344$, $P = 0.082$, data not shown). Given that the study covered a large region, we performed the same test on log-transformed geographical distances. These yielded weakly significant correlations between genetic similarity and geographic distance (Mantel test: r_M full data set $= -0.4027$, $P = 0.046$; r_M reduced data set $= -0.389$, $P = 0.045$, data not shown). Low genetic differentiations between populations were also reflected in the neighbor-joining tree, which showed no clear pattern of population relationships. Accordingly, bootstrap analysis did not support significantly our population groupings (results therefore not shown).

Values of average gene diversity ranged between 0.14 and 0.26 (full data set) and 0.16 and 0.26 (reduced data set) respectively, and did not show any correlation to sample size (full data set: Pearson correlation $r_P = 0.03$, $P = 0.46$; reduced data set $r_P = -0.35$, $P = 0.09$; Table 1). Average Soerensen dissimilarity among members of a given population yielded similar values between 0.17 and 0.45 (both full and reduced data set) and revealed comparable patterns, as the Pearson correlation coefficients between genetic and Soerensen diversity were $r_P = 0.68$ ($P = 0.002$, full data set) and $r_P = 0.77$ ($P < 0.001$, reduced data set). There were no differences between both diversity values for the FLMA populations, and neither those of the surrounding populations nor those of all natural habitats investigated differed (full and reduced data set, *t*-test, $P > 0.19$ in all cases).

Discussion

Our study is the first attempt known to us applying molecular methods to study impact on genetic consequences of colonization events of FLMAs in eastern Germany and elucidate potential dispersal routes of species into FLMAs. The comparative analysis of genetic differentiation within and among populations of *E. palustris* from FLMAs and natural habitats showed high within-population variability and only moderate genetic differentiation among populations. Genetic diversity values of FLMA populations were equally high as those of the natural habitats, and we found no indications of genetic bottlenecks causing reduction in the genetic diversity due to founder events for the mining populations.

Our results are in line with Mengoni et al. (2000) who reported equally high genetic diversity of *Silene paradoxa* L. populations from copper mining deposits and neighboring non-contaminated sites in central Italy. Krüger et al. (2002) as well as Brock et al. (2007) found similar or even higher values of genetic diversity e.g. for *Aster tripolium* L. in natural populations in comparison to anthropogenic salt-contained habitats. Reisch (2007) verified the rather surprising result of similar genetic diversity values for populations of *Saxifraga tridactylites* L. in natural and man-made habitats. Independent and/or successive colonization events from different populations, high rates of immigration, rapid population growth after founding and effective seed dispersal are predestined to explain similar levels of genetic diversity in natural and secondary habitats, in comparable extent as our findings. Moreover, all *E. palustris* populations studied were composed of genetically different individuals that contributed to genetic diversity. Although clonal growth is generally possible (Haas 2000; Kühn and Klotz 2002) this kind of reproduction does not play a major role after colonization, neither in the primary nor in the secondary habitats.

Evidence of separation between natural surrounding populations and those of FLMAs could be found neither in the PCoA nor in the neighbor-joining tree. Our results suggest that the populations of *E. palustris* in eastern Germany are well connected by gene flow.

Since seed dispersal (possible also for distant populations) as well as pollen flow (mainly within populations) might have a considerable impact on the genetic structure of *E. palustris*, we assume that both mechanism counteract genetic differentiation among populations (Brzosko and Wróblewska 2003; Lian et al. 2003). High levels of gene exchange and independent colonization from different populations explain weak genetic differentiation among populations (McCauley et al. 1995). Thus, the present genetic structure and weak overall population differentiation suggest that the colonization of FLMAs is due to several independent founder events from different source populations. In accordance with our findings are the data on the closely related species *Epipactis helleborine* (L.) Crantz that the colonization of secondary habitats in urban areas can be attributed to different founder individuals and events, instead of a single colonization and *in situ* propagation (Hollingsworth and Dickson 1997). Krüger et al. (2002) also failed to demonstrate a clear separation between populations of two salt-tolerant species, *Aster tripolium* L. and *Salicornia ramosissima* J.Woods from natural habitats and from anthropogenic salt-polluted sites caused by independent colonization from different populations.

Other studies on population differentiation of orchid species are contradictory to our findings. Wallace (2002) reports strong genetic differentiation and little genetic exchange between highly fragmented *Platanthera leucophaea* Lindl. populations while Brzosko and Wróblewska (2003) found low genetic differentiation ($F_{ST} = 0.017$) for populations of *Cephalanthera rubra* (L.) Rich. However, mean distances among populations in the latter study were only 500 m, which hardly seems to be a barrier for gene flow through pollen or seeds, as orchids are regarded as species with exceptionally high long-distance dispersal ability (Willems 1982; Ridley 1990; Bonn and Poschlod 1998).

Next to the estimation of genetic diversity and differentiation of *E. palustris* populations in FLMAs and natural habitats, potential dispersal routes of the species into FLMAs should be detected. However, in our study, it was impossible to define the source populations for the recently founded FLMA populations of *E. palustris* with two genetic marker methods. Our study species apparently preserves most genetic variability within populations, as shown in the PCoA ordination where all populations overlapped in the diagram (Fig. 2). The correlation between Soerensen similarity and geographic distances was not significant. The use of logarithmus-tranformed geographic distances however resulted in a weak but significant correlation between the genetic and geographic distances supporting the presumption of a colonization of FLMAs predominantly but perhaps not exclusively from close surroundings. In addition, also the low resolution of the neighbor-joining tree supports the idea of repeated independent colonization events of the FLMAs from the near surroundings (Krüger et al. 2002; Reisch 2007). Given that the Mantel test did not indicate strong effects of geographic distance, events of larger distance dispersal cannot be excluded by our data, and FLMAs might be reached by individuals from populations farther than 125 km away.

We may conclude that FLMAs play a very important role in fragmented landscapes because many wind-dispersed species immigrate at higher than average frequencies into these areas (Klotz et al. 2000), later dominating in pioneer habitats and early succession stages (Bakker et al. 1996; Fort and Richards 1998). This is particularly important for threatened plant species, among them many orchids (Heyde and Krug 2000; Kirmer et al. accepted). Although colonization after long-distance dispersal often leads to genetic impoverishment due to founder events and genetic bottlenecks (Gustafsson 2000; Cozzolino et al. 2003; He et al. 2004), we found no evidence for this phenomenon in *E. palustris,* as measures of genetic diversity were similar in FLMAs and in the

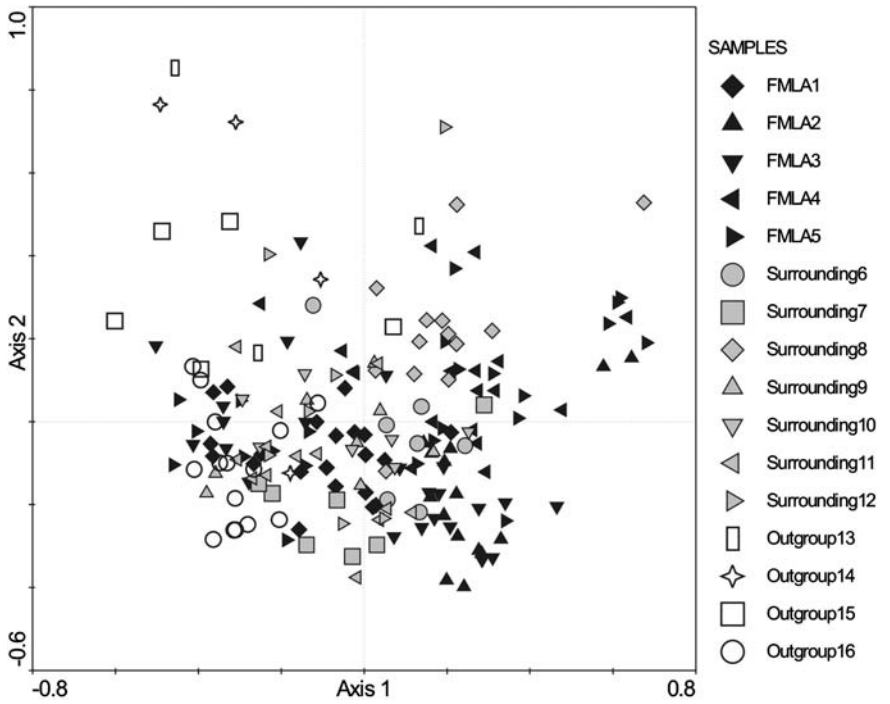

Fig. 2 Principal Coordinate Analysis (PCoA) of the combined miRPF and RAPD data (full dataset). Variance explained by the first axes was relatively low (Axis 1: 7.4%; A 2: 5.0%; A 3: 4.4%, A 4: 3.5%) indicating the absence of a simple genetic structure (PCoA based on square-root transformed Soerensen distance)

surrounding primary habitats. Thus, our data indicate that FLMAs could act as areas of pronounced genetic diversity. FMLAs may thus fulfill an important function as refugia and stepping-stones in a landscape otherwise lacking suitable habitats for endangered plants. However, colonization via spontaneous succession will be only successful if valuable ecological characteristics of FLMAs such as availability of open space (increasing light availability) and low-nutrient sites (decreasing nitrogen availability) are not destroyed by recultivation methods such as leveling of the surface, ameliorating of nutrient-poor substrates, and seeding or planting of species not suited to the present habitat conditions. Therefore, in FLMAs suitable priority areas for nature conservation should be reserved where site conditions are not altered by these restoration methods.

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