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ORIGINAL PAPER

Sexual reproduction in two mixed stands of coastal and interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in Germany

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Abstract

Adult individuals and seeds of two mixed stands of coastal and interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were analysed for genetic differentiation between the two varieties and evidence of intervarietal pollination. Clear genetic discrimination between the two varieties was observed based on multilocus evaluation of nine microsatellite markers using other Douglas-fir stands of known variety composition for comparison. Analysis of pollination distances showed that 80% of pollinations took place within a distance of about 44–55 m. Analysis of stand structure showed clearly separated areas of mainly coastal or interior Douglas-fir within both stands. Together with short pollination distances this led to an apparent dominance of intravarietal pollinations. However, analysis of pollination partners of trees growing near the border of the variety specific areas, does not indicate the existence of reproductive barriers between trees of the two varieties growing in mixed stands. Therefore, commercial seed harvesting in mixed stands should be avoided if the production of seed lots of pure coastal or interior Douglas-fir is intended.

Keywords Douglas-fir · SSR · Variety discrimination · Sexual reproduction · Intervarietal mating

Introduction

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a conifer native to western North America. Mainly two different varieties have been described, the coastal "green" variety (*Pseudotsuga menziesii* var. *menziesii* (Mirbel) Franco) growing on the North American Pacific Coast of Canada and the US, and the interior "blue" variety (*Pseudotsuga menziesii* var. *glauca* (Mayr) Franco), its range following the Rocky Mountains from Canada to Mexico (Aas 2008). The "grey" or Fraser River Douglas-fir (*Pseudotsuga menziesii* var. *caesia* (Schwer.) Franco), has been described as a third intermediate variety but this is not commonly accepted (Kleinschmit and Bastien 1992; Spellmann et al. 2015). Based on genetic analyses, the geographically isolated Mexican Douglas-fir (not used in European Forestry) is also considered as a separate variety (Gugger et al. 2011; Wei et al. 2011).

Douglas-fir shows a high morphological variety across its native range in North America. In general, green/coastal and blue/interior Douglas-fir can be morphologically differentiated by foliage colour and cone morphology. The needles of the green/coastal variety tend to be longer and have a yellowish to dark green colour. Cones are 6–11 cm long. The characteristic trident bracts tend to be straight appressed. The blue/interior variety has shorter needles with greyish to blueish green colour. Cones are 4–8 cm long, the bracts tend to be spreading or reflexed (Aas 2008).

Genetic differentiation of both varieties has been analysed using isoenzyme variation patterns. Li and Adams (1989) analysed 104 Douglas-fir populations using 20 different isoenzyme markers, and found clearly separated clusters corresponding to the two different varieties. Isoenzyme analysis has been applied for variety discrimination in multiple studies (see Fussi et al. (2013)). Later, the development of nuclear microsatellite markers (Slavov et al. 2004) allowed their application for

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variety discrimination and the detection of genetic variation within the natural ranges of both varieties (Hintsteiner et al. 2018; Neophytou et al. 2020; van Loo et al. 2015). The cited studies showed a clear genetic separation between the green/coastal and blue/interior varieties based on 13 SSR markers.

Both varieties are genetically compatible. However, large parts of their distribution ranges are separated. The contact zone of both varieties lies in the northern part of their natural ranges in Southern Canada (Aas 2008). Analysis of maternally inherited mitochondrial and paternally inherited chloroplast DNA sequences of both varieties showed a quite clear separation between the different mitotypes of both varieties. Chloroplast haplotypes, however, were mixed in the contact zone of both varieties, indicating a history of pollen transfer between the two varieties (Gugger et al. 2010). Successful artificial crosses between both varieties have been reported (Adams and Stoehr 2013; Orr-Ewing et al. 1972) and intervarietal hybrids show a high potential for growth and cold hardiness (Braun 1999; Rehfeldt 1977).

Douglas-fir was introduced to Europe about 190 years ago (Kownatzki 2011; Spellmann et al. 2015). In Germany, cultivation started around the year 1850 (Kownatzki 2011; Spellmann et al. 2015). And according to data from the last national forest inventory, Douglas-fir nowadays represents the most frequent non-native forest tree species in Germany, covering about 2% of the German forest area (Riedel et al. 2017). Most German Douglas-fir belongs to the green/coastal variety, which performed better in European provenance field tests (Kleinschmit and Bastien 1992; Konnert and Ruetz 2006; Spellmann et al. 2015). Hermann and Lavender (1999) list the interior/blue variety as generally unsuitable for cultivation in Europe. And for Bavaria, Konnert and Ruetz (2006) recommend that seed harvest should only be carried out in stands containing trees of the coastal type. Still, due to its higher frost tolerance, the blue/interior variety might be better adapted to winter frosts in some more continental or mountainous regions (Bastien et al. 2013). Unfortunately, the blue/interior variety is more susceptible to needle cast caused by *Rhabdocline pseudotsugae* (Spellmann et al. 2015). All this highlights the importance of keeping both varieties separated in the production of forest reproductive material unless the production of hybrids is explicitly planned.

The aim of this study was to investigate genetic variety discrimination and the reproductive relations between the two varieties growing in close neighbourhood. Do both varieties growing in mixed stands mate randomly under natural conditions, or do reproductive barriers between the two varieties lead to the formation of two more or less separated reproductive clusters? What consequences for commercial seed harvest in mixed stands can be drawn.

Material and methods

Plant material

Two different mixed stands of Douglas-fir were chosen for further analysis. Selection of the two stands was based on the availability of seeds for offspring analysis.

The stand Mitterfels is an approved seed stand (category: selected, European Council Directive 1999/105/EC) located in Bavaria (48°57′49"N 12°34′42"E). It consists of 60 trees of Douglas-fir, growing on 0.6 hectares (ha). Stand age at the time of sampling was 111 years. 225 seeds from a single tree harvest of 27 trees (1–10 seeds per tree) and 4 offspring individuals from natural regeneration were analysed. Geographic positions of all trees were measured using a handheld GPS receiver (Garmin eTrex® 10, Garmin, Schaffhausen, Switzerland, precision ± 3 m).

The stand Mirow, also an approved seed stand (category: selected, European Council Directive 1999/105/EC), is located in Mecklenburg-West Pomerania (53°12′54"N 12°54′03"E). It consists of about 297 trees of Douglas-fir, growing on 2.1 ha (mixed with a few individuals of beech and pine). Stand age at the time of sampling was 76 years. 510 seeds from a single tree harvest of 24 trees (4- 30 seeds per tree) were analysed. Relative positions (distance and angle) of 240 trees were measured using a LaserAce 1000 rangefinder (Trimble, Sunnyvale, California, USA).

Information about the origin (seed source) of the plant material used for stand establishment was not available. In Mitterfels, according to information of the local forester and based on prior phenotype assessment, green/coastal Douglas-fir trees were planted in the Eastern part of the stand and blue/interior Douglas-fir trees were planted in the Western part. For the stand Mirow, no information about the planting scheme was available. Phenotypic assignment of the trees to the different varieties (mainly based on foliage colour) proved to be impossible from the ground. For the trees used for seed harvest cone morphology with either straight appressed or spreading/reflexed bracts was recorded. Diameter at breast height (DBH) of all trees was recorded.

DNA extraction and SSR marker analysis

For adult trees or natural regeneration, DNA was extracted from needle or cambium samples. For the genetic analysis of the seeds, embryos were extracted from the seeds and used for DNA extraction. DNA extraction was carried out according to the protocol of Dumolin et al. (1995). Genotyping was performed as described in Wojacki et al. (2019), using 9 microsatellite (SSR) markers (Slavov et al. 2004), PmOSU3D5, PmOSU4A7; PmOSU3B2, PmOSU1F9, PmOSU2G12, PmOSU3G9, PmOSU3F1, PmOSU3B9 and PmOSU2D4) in two multiplex sets. The softwares GeneMarker V3.0.0 (SoftGenetics LLC, State College, PA, USA) and Genome Lab V 10.2.3 (Sciex, Framingham, MA, USA) were used for allele calling.

Data analysis

The software STRUCTURE (Pritchard et al. 2000) which uses a Bayesian clustering approach, was used for analysis of genetic structures present in the analysed stands. The mean membership coefficients for a given number of genetic clusters (K) with K ranging from $K=1$ to $K=10$ at 20 runs per K were calculated, using the following parameters: no admixture, correlated allele frequencies, 20,000 burn-in replications, 20,000 Markov Chain Monte Carlo (MCMC) replications after burn-in. Results of the individual runs per K were merged using the web tool CLUMPAK (Kopelman et al. 2015). The optimal number of genetic clusters was estimated with the ΔK method described in Evanno et al. (2005). The webtool Structure Harvester (Earl 2012) was used for determination of the optimal ΔK. The web tool STRUCTURE PLOT (Ramasamy et al. 2014) was used for graphical display of the results.

The software COLONY (Version 2.0.6.5 and 2.0.6.6., Jones and Wang 2010) was used for pedigree reconstruction. Rates of drop outs/null alleles and mistyping were estimated by COLONY and adjusted separately for each stand. Analyses were run using the following parameters: female and male polygamy, monoecious and diploid species, known maternal sibship of seeds/offspring individuals sampled from identical seed parents, length of run: medium, analysis method: FL (full likelihood)-PLS (pairwise-likelihood score) combined, weak prior. All other parameters were set to default. To verify the results, the analysis was repeated at least three times for each stand using different random seed numbers.

Results and discussion

Structure analysis was carried out using the SSR data of the adult individuals of the two analysed mixed stands (both supposed to consist of a mixture of the coastal/green and interior/blue varieties) and—for comparison—the data of the adult individuals of four seed orchards described in Pakull et al. (2021) (three consisting of the coastal/green variety and one declared to consist of "grey" variety (*Pseudotsuga menziesii* var. *caesia* (Schwer.) Franco) and four seed stands described in Wojacki et al. (2019) (all four consisting of coastal/green Douglas-fir). Bar plots of K2, K3 and K7 are shown in Fig. 1.

Structure analysis shows a clear separation between the green/coastal and blue (or "grey")/interior varieties. This clear genetic discrimination between the two varieties is in accordance with prior results based on isoenzyme or SSR marker analysis (see Fussi et al. (2013) and citations within). At $K=2$ all three seed orchards and all four seed stands consisting of green/coastal variety Douglas-fir are—with few exceptions—assigned to one cluster (shown in green in Fig. 1) and the seed orchard declared to consist of "grey" Douglas-fir is assigned to another cluster (shown in blue in Fig. 1). Most individuals of the mixed stands are assigned to either the one or the other cluster. The exceptional individuals within the green/coastal seed orchards and seed stands, which are assigned to the blue/interior cluster are either based on imperfect assignment or on admixture of some individuals of the other variety during stand establishment, which cannot be ruled out completely. Most individuals of the mixed stands (Mitterfels 90.0%, Mirow 79.5%) are assigned to one cluster with $P>0.95$. Individuals with no clear assignment to a cluster ($P<0.7$) only count up to 3.33% (Mitterfels) and 5.1% (Mirow) of all individuals.

Even though $K=2$ shows by far the highest ΔK (data not shown), looking at the other results shows that at $K=3$ the green cluster is separated into 2 subclusters while the blue cluster remains more or less unchanged. Some individuals of the green/coastal seed orchards and seed stands, that were assigned to the blue cluster at $K=2$, are now assigned to one of the two green subclusters, leading to fewer exceptions from the otherwise mostly consistent composition. The blue cluster remains more or less undivided up to $K=7$. This indicates that the separation between the two varieties seems to be very strong. But it also shows that the genetic composition of the blue/"grey" variety Douglas-fir trees analysed here seems to be quite homogenous. Based on the available data, it cannot be determined, whether this is characteristic for the blue/interior variety in general, or if it is based on a similar genetic ancestry of the blue/interior variety plant material used for stand (and seed orchard) establishment. Since the green/coastal variety is predominant in Germany, the amount of imported seed material of the blue/interior variety in general could be limited, leading to an artificial founder effect. Eckhart et al. (2017) detected a general lower genetic diversity in seedlings from European compared to North American seed stands, but found only few individuals of the interior/blue variety within the analysed European seed stands.

Noticeable is the fact that the green/coastal variety individuals within the stand Mirow also seem to be genetically quite homogenous, most of them are assigned to the same subcluster even at K7. This is probably due to the seed/plant material used for stand establishment.

A hierarchical STRUCTURE analysis using only the individuals assigned to the blue or green cluster ($P>0.7$),

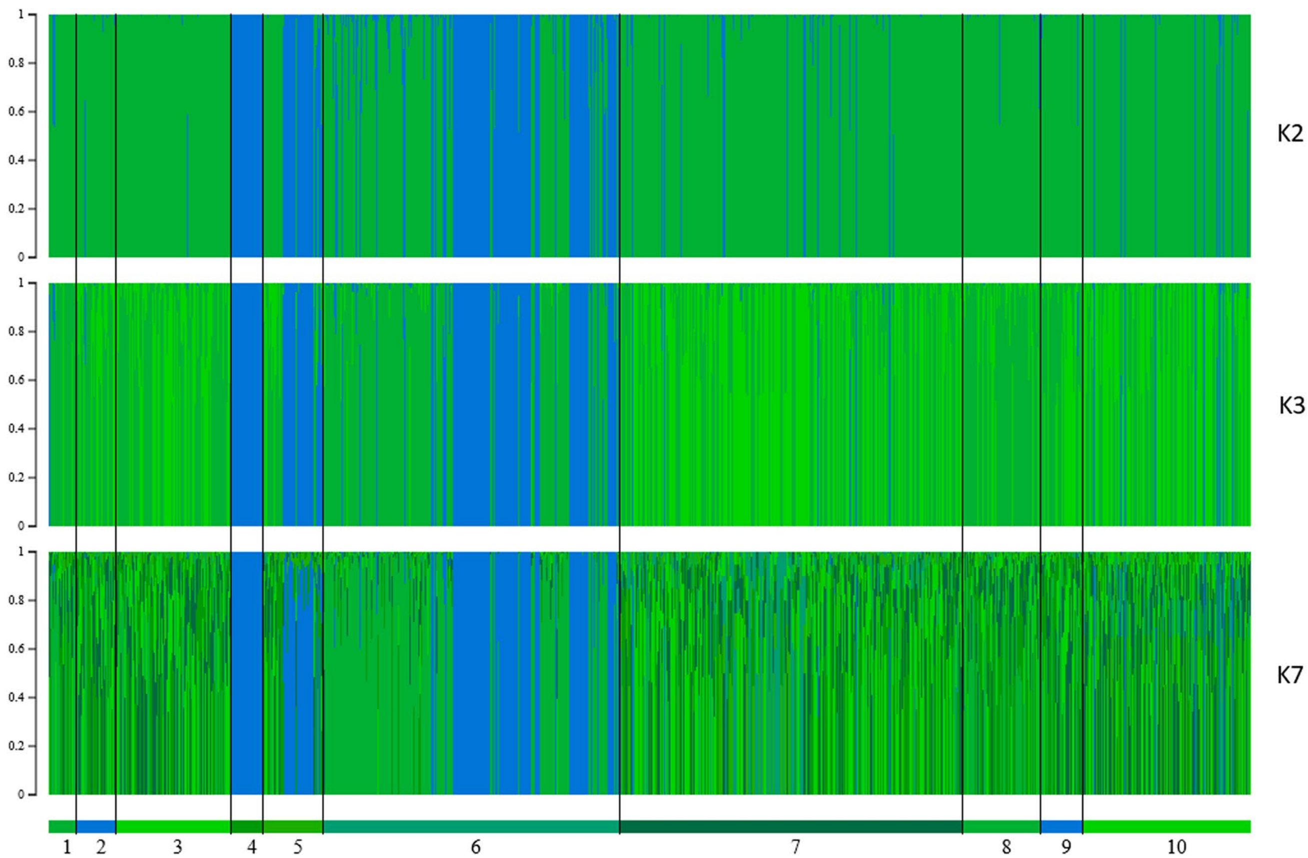

Fig. 1 Structure analysis: bar plots representing mean membership coefficients for the major modes for $K=2$, $K=3$ and $K=7$ merged with CLUMPAK (Kopelman et al. 2015), drawn with STRUCTURE PLOT (Ramasamy et al. 2014). Populations: 1 = seed orchard Voigtsdorf (green); 2 = seed orchard Niederfinow (green); 3 = seed orchard Harsefeld (green); 4 = seed orchard Beerwalde ("grey", see Pakull et al. (2021) for seed orchard description); 5 = mixed stand Mitterfels; 6 = mixed stand Mirow; 7 = seed stand Chorin (green), 8 = seed stand Morschen (green); 9 = seed stand Drebkau (green); 10 = seed stand Romrod (green, see Wojacki et al. (2019) for seed stand description). Cluster 2 corresponds to the blue variety and is shown in blue, Cluster 1 and 3–7 correspond to different subclusters of the green variety and are shown in different shades of green

respectively, lead to no other remarkable results (data not shown).

When combining the cluster assignment of the individual trees of the mixed stand with the information on the geographic positions (GPS or relative positions, Figs. 2 and 3), both stands show a clear separation of areas with mainly blue cluster Douglas-fir trees (interior variety) and areas with mainly green cluster Douglas-fir trees (coastal variety). In the stand Mitterfels, this corresponds to the stated planting scheme, with the green/coastal Douglas-fir trees growing in the Eastern part of the stand and the blue/interior Douglas-fir trees growing in the Western part. For the stand in Mirow, no previous information about the positions of the different varieties in the stand was available. Exceptional trees growing in the "wrong" area did not show a noticeable lower DBH. These trees thus do not represent younger trees established by natural regeneration, but indicate either imperfect cluster assignment or intermixture of some individuals of the other variety during stand establishment. Parentage analysis was successful for 99.2% (Mirow) to 98.3% (Mitterfels) of all analysed offspring individuals. Selfing rates ranged on low levels between 2.5 (Mirow) and 3% (Mitterfels). Pollination from unknown pollinators from outside of the stands ranged between 10.5% (Mitterfels) and 23.7% (Mirow). According to the local forest maps both stands have Douglas-fir growing in the neighbourhood. For Mirow, no closely located Douglas fir stands are listed but single Douglas-fir trees are growing in a number of adjacent forest parcels (1–10 trees per parcel). For Mitterfels, the nearest Douglas fir stands are located in 1100 m (southward) and 1350 m (northward).

Parentage analysis showed that in both analysed stands blue cluster Douglas-fir trees were mainly pollinated by blue cluster Douglas-fir trees. Of all analysed offspring from blue cluster seed parents (offspring from selfings, unknown or not clearly assigned pollinators excluded, $N=125$ in Mitterfels and $N=182$ in Mirow) 93.6% (Mitterfels) and 76.9% (Mirow) had blue cluster pollen donors. Green cluster

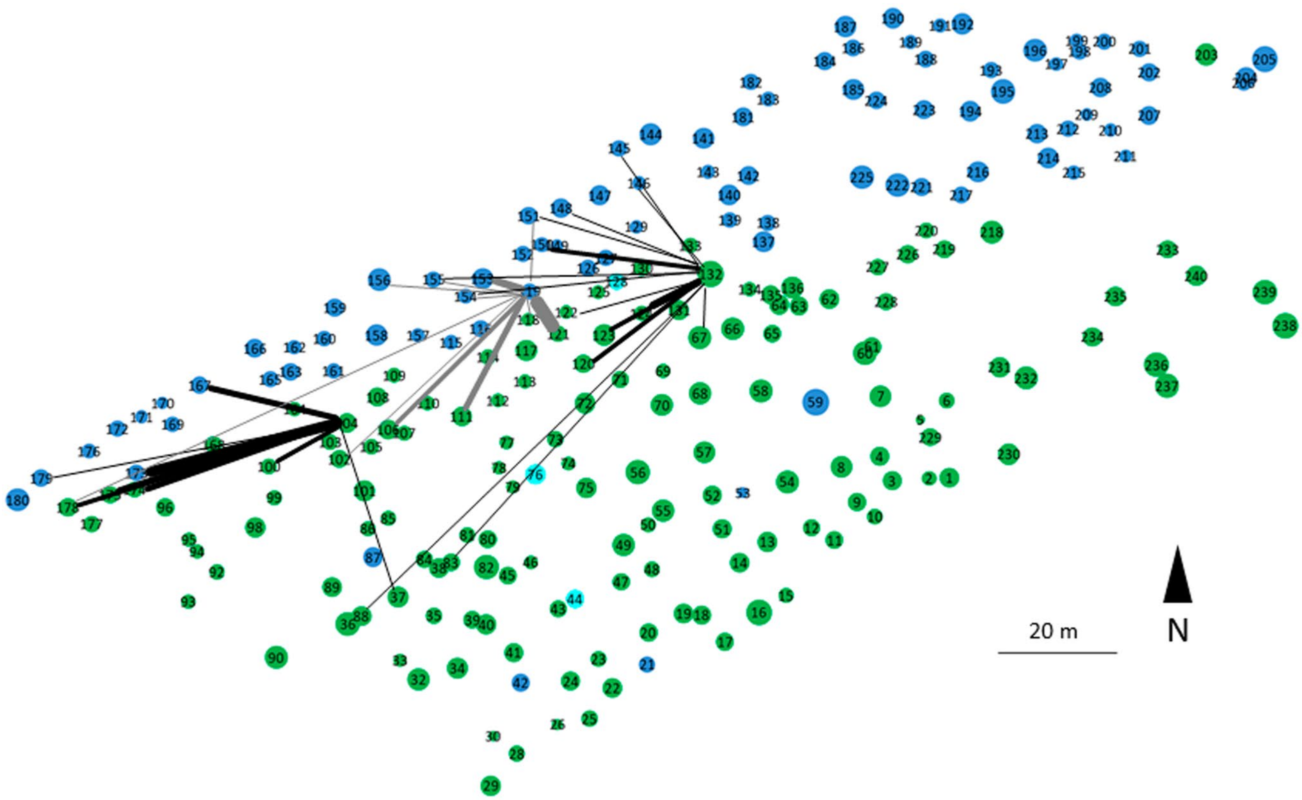

Fig. 2 Spatial positions of 240 trees of the stand Mirow. Each dot represents a tree. Dot size varies in relation to determined DBH (not to scale with tree distance). Dot colour indicates STRUCTURE cluster assignment at $K=3$ (cluster 2: blue, cluster 1 and/or 3: green, unclear cluster assignment ($P<0.7$): turquoise). Pollination partners of three trees used for seed harvest (104, 119, 132) are represented by lines drawn between seed and pollen parent. Thickness of the lines varies in relation to the number of observed pollinations

Douglas-fir trees were mainly pollinated by green cluster Douglas-fir trees. Of all analysed offspring from green cluster seed parents ($N=45$ in Mitterfels and $N=181$ in Mirow) 73.3% (Mitterfels) and 83.4% (Mirow) had green cluster pollen donors. This could indicate some kind of reproductive barrier between the two different varieties of Douglas-fir.

However, when combining the results of parentage analysis with information on the spatial positions of the individual trees, and using this information to calculate pollination distances (Fig. 4), it can clearly be seen that most pollination events take place within short distances. 80% of all pollinations takes place within a radius of about 44 (Mirow)–55 m (Mitterfels) around the seed tree. Other studies measuring pollen dispersal or pollination distances for Douglas-fir also report that the majority of all pollen is dispersed within distances of about 50 m or even less (Erickson and Adams 1989; Prat 1995; Silen 1962; Wojacki et al. 2019). Due to the separated areas, in which green/coastal and blue/interior cluster Douglas-fir trees are growing within the analysed stands, the majority of mating partners in the respective distance will belong to the same variety. Dominance of same variety pollinations thus seems to be based on stand structure and not on some kind of reproductive barrier.

To check for other evidence for a potential reproductive barrier between the different varieties, the pollination partners of those trees used for seed harvest and growing near the border between the two different areas in the stand Mirow were analysed in more detail. In Fig. 2, the pollination events of three trees (104 (green), 119 (blue), 132 (green)) used for seed harvest are represented by lines drawn between seed and pollen parent. This likely illustrates the influence of wind direction on pollination. Most pollination partners are located in western to southwestern positions to the seed parent. Since the thus probably predominating western and southwestern winds carried pollen from the blue/interior variety-dominated area of the stand to the green/coastal variety-dominated area of the stand, the seed parents growing in the border region were likewise pollinated by green and blue mating partners (tree 104 (green): 47.4% of known pollen donors belong to the blue/interior variety and 52.6% belong to the green/coastal variety; tree 119 (blue): 33.3% blue and 66.7% green pollen donors; tree 132 (green): 40% blue and 69% green pollen donors). Hence, the spatial mating patterns in the border region between the two different varieties growing in the stand Mirow do not indicate reproductive

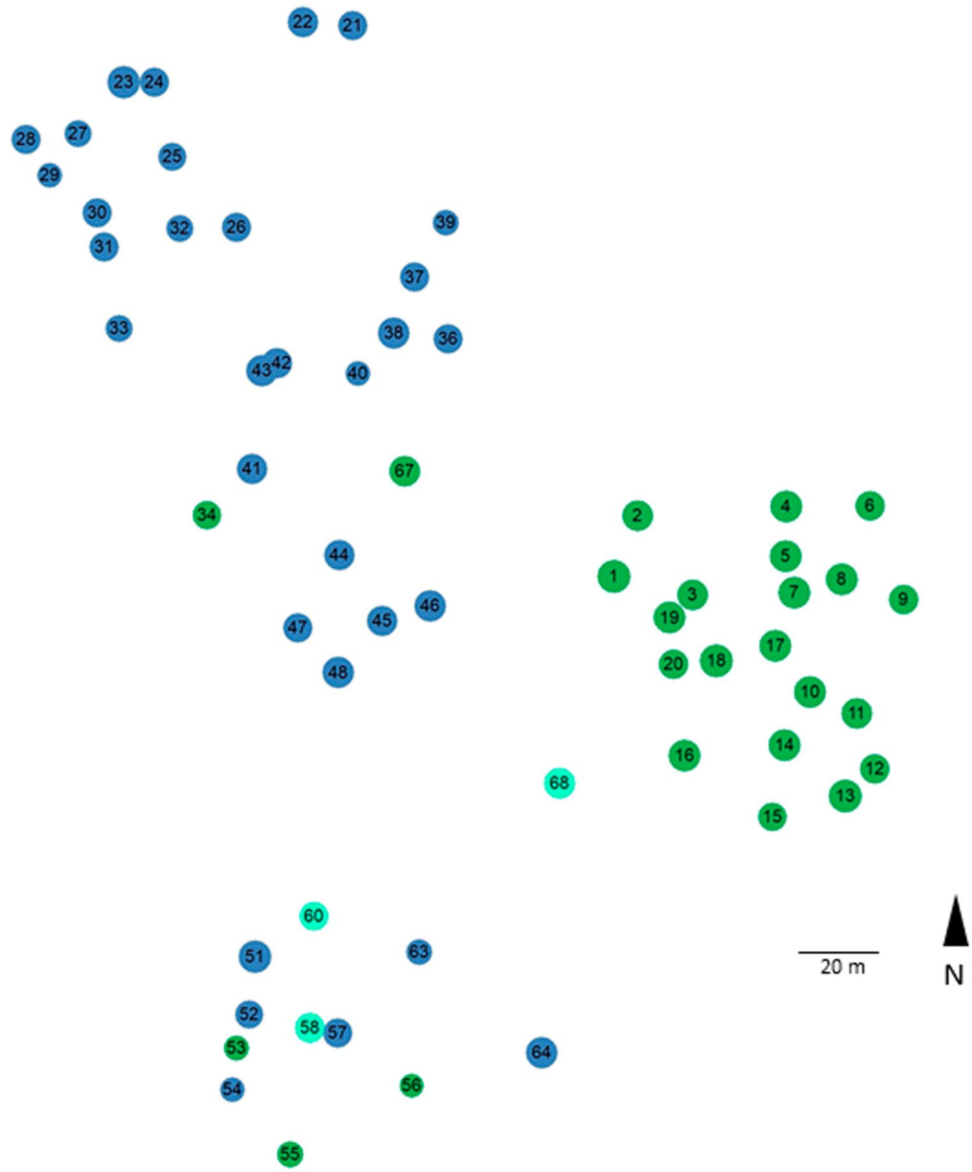

Fig. 3 Spatial positions of 60 trees of the stand Mitterfels. Each dot represents a tree. Dot size varies in relation to determined DBH (not to scale with tree distance). Dot colour indicates STRUCTURE cluster assignment at $K = 3$ (cluster 2: blue, cluster 1 and/or 3: green, unclear cluster assignment ($P < 0.7$): turquoise)

barriers between the two varieties. Flowering times of both varieties must have been overlapping.

Artificial crossings between both varieties have been reported (Adams and Stoehr 2013; Orr-Ewing et al. 1972) which shows general interfertility of both varieties and mixed chloroplast haplotypes in the contact zone of both varieties indicate a history of pollen transfer between both varieties (Gugger et al. 2010). This study indicates that intervarietal mating in mixed stands of both varieties seems to be possible without any reproductive barriers.

Conclusions

The multilocus genetic data provided by the used marker set allowed a clear genetic differentiation between the two different Douglas-fir varieties and successful parentage analysis. Stand structure and relatively short pollination distances lead to an apparent dominance of intravarietal pollinations. However, analysis of trees near the borders of the variety specific areas does not indicate the existence

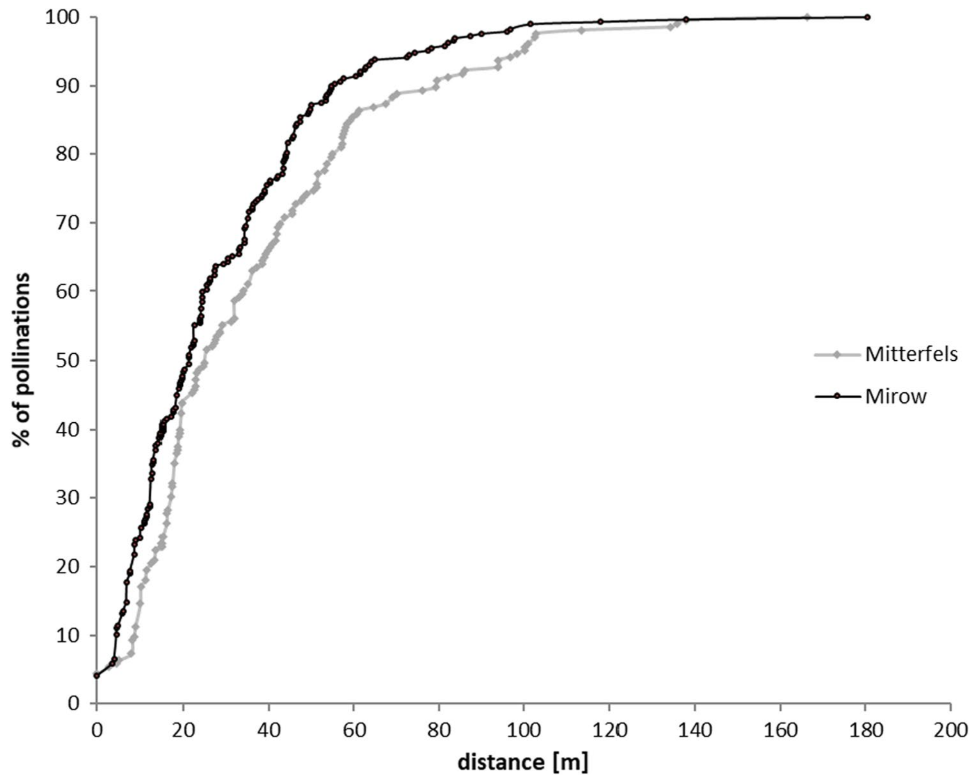

Fig. 4 Pollination distance, percentage of pollination events within a certain distance between seed and pollen parent

of reproductive barriers between the two varieties. Commercial seed harvesting in mixed stands should therefore be avoided if the goal is to obtain seed lots of pure coastal or interior Douglas-fir.

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Author contributions BP analysed the data and wrote the manuscript. JW designed and performed the sampling activities and was involved in genotype analysis. PE helped with data analysis and writing of the manuscript. BF was involved in stand selection and data collection. DA carried out the practical analyses in the laboratory. HL was responsible for study conception and design. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Upon acceptance for publication of the manuscript all data on SSR genotypes will be made available on OSF (Open Science Framework, https://osf.io).

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Consent to participate All authors agreed to participate in the described study.

Consent for publication All authors agreed to publish the results described in this manuscript.

Ethical approval Not applicable.

References

Aas G (2008) Die Douglasie (*Pseudotsuga menziesii*) in Nordamerika: Verbreitung, Variabilität und Ökologie. LWF Wissen 59:7–12

Adams RP, Stoehr M (2013) Multivariate detection of hybridization using conifer terpenes II: analyses of terpene inheritance patterns in *Pseudotsuga menziesii* F1 hybrids. Phytologia 95:42–57

Bastien J-C, Sanchez L, Michaud D (2013) Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Forest tree breeding in Europe (Springer), Dordrecht

Braun H (1999) Douglas-fir breeding in Saxony. Silvae Genetica 48:69–77

Dumolin S, Demesure B, Petit R (1995) Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. Theor Appl Genet 91:1253–1256

Earl DA (2012) Structure harvester: a website and program for visualizing structure output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Eckhart T, Walcher S, Hasenauer H, Van Loo M (2017) Genetic diversity and adaptive traits of European versus American Douglas-fir seedlings. Eur J Forest Res 136:811–825

Erickson V, Adams W (1989) Mating success in a coastal Douglas-fir seed orchard as affected by distance and floral phenology. Can J for Res 19:1248–1255

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620

Fussi B, Konnert M, Dounavi A (2013) Identification of varieties and gene flow in Douglas fir exemplified in artificially established stands in Germany. Ann for Res 56:249–268

Gugger PF, Sugita S, Cavender-Bares J (2010) Phylogeography of Douglas-fir based on mitochondrial and chloroplast DNA sequences: testing hypotheses from the fossil record. Mol Ecol 19:1877–1897

Gugger PF, González-Rodríguez A, Rodríguez-Correa H, Sugita S, Cavender-Bares J (2011) Southward Pleistocene migration of Douglas-fir into Mexico: phylogeography, ecological niche modeling, and conservation of 'rear edge' populations. New Phytol 189:1185–1199

Hermann RK, Lavender DP (1999) Douglas-fir planted forests. New for 17:53–70

Hintsteiner WJ, van Loo M, Neophytou C, Schueler S, Hasenauer H (2018) The geographic origin of old Douglas-fir stands growing in Central Europe. Eur J Forest Res 137:447–461

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555

Kleinschmit J, Bastien J (1992) IUFRO's role in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) tree improvement. Silvae Genetica 41:161–173

Konnert M, Ruetz W (2006) Genetic aspects of artificial regeneration of Douglas-fir (*Pseudotsuga menziesii*) in Bavaria. Eur J Forest Res 125:261–270

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15:1179–1191

Kownatzki D (2011) Zum Douglasienanbau in Deutschland. Landbauforschung 344:5645

Li P, Adams W (1989) Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). Can J for Res 19:149–161

Neophytou C, van Loo M, Hasenauer H (2020) Genetic diversity in introduced Douglas-fir and its natural regeneration in Central Europe. For.: Int J for. Res 93:535–544

Orr-Ewing A, Fraser A, Karlsson I (1972) Interracial crosses with douglas fir: early field results. Brit Columbia Forest Serv Res Notes 55:33

Pakull B, Eusemann P, Wojacki J, Ahnert D, Liesebach H (2021) Genetic diversity of seeds from four German Douglas fir (*Pseudotsuga menziesii*) seed orchards. Eur J Forest Res 140:1543–1557

Prat D (1995) Mating system in a clonal Douglas fir (*Pseudotsuga menziesii* (Mirb) Franco) seed orchard. II. effective pollen dispersal. Ann Sci for 52:213–222

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using Multilocus genotype data. Genetics 155:945

Ramasamy RK, Ramasamy S, Bindroo BB, Naik VG (2014) Structure plot: a program for drawing elegant STRUCTURE bar plots in user friendly interface. Springerplus 3:1–3

Rehfeldt G (1977) Growth and cold hardiness of intervarietal hybrids of Douglas-fir. Theor Appl Genetics 50:3–15

Riedel T, Henning P, Kroiher F, Polley H, Schmitz F, Schwitzgebel F (2017) Bundeswaldinventur (BWI 2012) Inventur und Auswertemethoden, pp. 1–124. https://www.bundeswaldinventur.de/fileadmin/SITE_MASTER/content/Downloads/BWI_Methodenband_web.pdf

Silen RR (1962) Pollen dispersal considerations for Douglas-fir. J Forest 60:790–795

Slavov GT, Howe GT, Yakovlev I, Edwards KJ, Krutovskii KV, Tuskan GA, Carlson JE, Strauss SH, Adams WT (2004) Highly variable SSR markers in Douglas-fir: Mendelian inheritance and map locations. Theor Appl Genet 108:873–880

Spellmann H, Weller A, Brang P, Michiels H-G, Bolte AT (2015) Douglasie (*Pseudotsuga menziesii* (Mirb.) Franco) in Potenziale und Risiken eingeführter Baumarten. Universitätsverlag Göttingen, Göttingen

van Loo M, Hintsteiner W, Pötzelsberger E, Schüler S, Hasenauer H (2015) Intervarietal and intravarietal genetic structure in Douglas-fir: nuclear SSR s bring novel insights into past population demographic processes, phylogeography, and intervarietal hybridization. Ecol Evol 5:1802–1817

Wei X-X, Beaulieu J, Khasa DP, Vargas-Hernández J, López-Upton J, Jaquish B, Bousquet J (2011) Range-wide chloroplast and mitochondrial DNA imprints reveal multiple lineages and complex biogeographic history for Douglas-fir. Tree Genet Genomes 7:1025–1040

Wojacki J, Eusemann P, Ahnert D, Pakull B, Liesebach H (2019) Genetic diversity in seeds produced in artificial Douglas-fir (*Pseudotsuga menziesii*) stands of different size. For Ecol Manage 438:18–24