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WOHER STAMMEN DIE ALTEN MITTELEUROPÄISCHEN DOUGLASIEN-BESTÄNDE?

Dissertation
zur Erhaltung des Doktoratsgrades (Dr. nat. techn.)
auf der Universität für Bodenkultur (BOKU), Wien

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Wien, September 2018

Vorwort und Danksagung

Die vorliegende Doktorarbeit ist eine kumulative Dissertation und beschäftigt sich mit der Herkunftsanalyse europäischer Douglasien-Altbestände. Die Arbeit selbst baut auf drei individuellen „peer-reviewed“ Publikationen auf. Diese befinden sich in Appendix 9.1 bis 9.3. Das Format der Publikationen variiert aufgrund unterschiedlicher Anforderungen der jeweiligen Zeitschriften. Die spezifisch angewandten Methoden, die Ergebnisse und Diskussionen können detailliert in den jeweiligen Kapiteln der Publikationen im Appendix nachgelesen werden.

Zitierungen dieser Arbeit sollten unter „Hintsteiner W. (2018) Herkunftsbestimmung europäischer Douglasien-Altbestände. Dissertation. Universität für Bodenkultur, Wien, Seite n“ oder unter Bezugnahme auf die jeweilige Publikation im Speziellen vorgenommen werden.

Ich möchte diese Stelle nutzen, um vor allem jenen Personen und Institutionen zu danken, die mir diese Arbeit ermöglicht haben. Deshalb darf ich an erster Stelle meinen Betreuer, Rektor Univ.- Prof. DI Dr. Hubert Hasenauer, nennen. Prof. Hasenauer war mir ein wertvoller Mentor, Ratgeber, Beistand und auch Freund. Ich möchte Ihm deshalb sehr herzlich für die Möglichkeit eine Dissertation zu schreiben, für seine Geduld und für seinen Glauben an mich danken.

Ebenfalls ein großer Dank gebührt meinen beiden Arbeitgebern während der Erhebungen und Auswertungen der Dissertation, der Universität für Bodenkultur in Wien und der Alps-GmbH in Innsbruck. Die Zeit an diesen Institutionen hat mir sehr viele Erfahrungen für mein weiteres Leben gebracht und auch die Weichen für meine weitere berufliche Laufbahn gestellt.

Stellvertretend für alle Personen, die zum Gelingen dieser Arbeit beigetragen haben, möchte ich mich bei Dr. Charalambos Neophytou und bei Dr. Marcela Van Loo bedanken. Von ihren Erfahrungen konnte ich sehr profitieren.

Zu guter Letzt möchte ich von ganzem Herzen meiner Familie danken. Meine Frau Marion und meine beiden Töchter Valerie und Luise waren jene, die die größten Opfer während der Fertigstellung meiner Dissertation erbringen mussten. Sie hatten sehr viel Geduld mit mir und oftmals konnte ich Zeit nicht mit ihnen verbringen, sondern musste mich dieser Arbeit widmen. Sie haben mich aber immer ermuntert weiter zu machen und mir dadurch auch die Kraft gegeben durchzuhalten. Ich hoffe darauf, dass wir das Versäumte nachholen können.

Kurzzusammenfassung

Die Douglasie wurde erstmals im 19. Jahrhundert außerhalb ihres natürlichen Verbreitungsgebietes in Nordamerika nach Europa importiert. Überzeugt haben vor allem ihre hervorragenden Wuchseigenschaften. Doch folgten durch Importe von Saatgut aus verschiedenen Herkunftsgebieten auch Misserfolge im Douglasien-Anbau. Dies machte die Herkunftsfrage essentiell. Heutzutage wird die Douglasie aufgrund des prognostizierten Klimawandels und ihrer Trockenresistenz als eine mögliche Alternative zu unseren heimischen Baumarten in tieferen Lagen gehandelt.

In dieser Arbeit wurden deshalb 67 österreichische und deutsche Douglasien-Bestände hinsichtlich ihrer Herkunft untersucht. Diese Bestände sind zum Teil anerkannte Saatguterntebestände, welche sich natürlich verjüngen, eine gesamte Umtriebszeit repräsentieren und durch ihre hervorragenden Wuchsleistungen hervorstechen. Deshalb ist die Herkunftsfrage dieser Bestände für die europäische Forstwirtschaft äußerst bedeutend, da die genetische Qualität dieser Bestände garantiert werden muss. Aus diesem Grund wurden 38 Douglasien-Referenzbestände aus dem natürlichen Verbreitungsgebiet mittels 13 hochvariabler Mikrosatellitenloci, einer genetischen Analyse und hierarchischen Clusterung anhand einer Bayesian-Methode in der Software STRUCTURE unterzogen, um einerseits die Phylogeographie, und andererseits die genetische Struktur dieser Art zu untersuchen. Die Ergebnisse waren Basis für die Entwicklung einer hierarchischen Zuordnungsmethode der 67 österreichischen und deutschen Bestände zu einem bestimmten Herkunftsgebiet, wobei das oberste hierarchische Level aus den beiden Varietäten und das unterste aus den Referenzpopulationen bestand. Der Großteil der untersuchten Bestände konnte der Küstenvarietät aus Zentral-Washington westlich des Kaskadenhauptkamms zugeordnet werden. Dieses Gebiet ist ident mit den Herkunftsempfehlungen für Mitteleuropa. Allerdings wurden einzelne Bestände auch Regionen außerhalb dieses Herkunftsgebietes zugeordnet, wie zum Beispiel Zentral-Oregon und Santa Fe (New Mexico), einem Gebiet der Inlandsdouglasie. Die Unsicherheit der Zuordnung nimmt in Richtung unterstes hierarchisches Level zu. Die Ergebnisse der Zuordnung waren auch Basis für die Untersuchung des Einflusses der Standortseigenschaften auf das Wachstum von Douglasien-Beständen gleicher Herkunft.

Abstract

Native to North America, the Douglas fir was first introduced to Europe in the 19th century because of its exceptionally good growth characteristics. There were, however, failures related to the cultivation of Douglas firs, primarily due to the importation of seeds from different regions, which underlines the importance of the question of origin. Today the Douglas fir is viewed as a possible alternative to indigenous tree species because of its resistance to drought, a potential result of climate change. The main purpose of this thesis was to investigate the origin of 67 Austrian and German Douglas fir populations. The majority of these populations comprises those accredited for seed harvesting, which are subject to natural rejuvenation and therefore represent a full rotation period and show outstanding growth characteristics. Since the genetic qualities of these populations need to be maintained, the question of their origin is of major importance for European forestry. 38 Douglas fir populations in their natural distribution area were genetically analysed by applying 13 highly variable microsatellite loci. Their hierarchical clustering was examined by applying the Bayesian-method with the software STRUCTURE to examine both the phylogeography and the genetic structure side by side. The data then served as a basis for the development of a hierarchical assignment method of the 67 Austrian and German populations concerning a specific origin of distribution. The highest hierarchical level consisted of two varieties, whereas the lowest level consisted of the reference population, thus resulting in the clear conclusion that the majority of the populations originates from the coastal variety found in Central Washington, west of the Cascade mountain range. This area is identical to the recommended origin for Central Europe. However, some of the populations could also be traced to regions beyond this area, for example Central Oregon and Santa Fe (New Mexico), an area of interior Douglas firs. The uncertainty concerning their assignment increases towards the lower hierarchical levels. The results of the assignment study served as a basis for investigating the influence of site characteristics on the growth of Douglas firs of the same origin.

Inhaltsverzeichnis

1 Einleitung 1
2 Zielsetzung und Arbeitsschritte 4
3 Daten 6
3.1 Datensatz I: Populationen aus dem natürlichen Verbreitungsgebiet 6
3.2 Datensatz II: Bestände unbekannter Herkunft 8
4 Vorgehensweise und Methodik 10
4.1 Analysekonzept 10
4.2 Genetische Struktur 11
4.3 Zuordnungsmethode 12
4.4 Herkunftsanalyse 12
5 Analyse und Ergebnisse 14
5.1 Genetische Struktur 14
5.2 Zuordnungsmethode 18
5.3 Herkunftsbestimmung 20
6 Diskussion 23
7 Schlussfolgerungen und Ausblick 26
8 Literaturverzeichnis 28
9 Appendix 33
9.1 Publikation I 33
9.2 Publikation II 34
9.3 Publikation III 35

1 Einleitung

Die Douglasie (*Pseudotsuga menziesii* (Mirb.) Franco) ist eine nordwest-amerikanische Nadelbaumart mit einem sehr großen Verbreitungsgebiet. Dieses reicht von 19° bis 55° nördlicher Breite und bis zu einem Meeresniveau von 3260 Metern Seehöhe (Hermann & Lavender 1990, Kleinschmidt & Bastien 1992). Innerhalb dieses Gebietes können grundsätzlich zwei Varietäten unterschieden werden. Es handelt sich dabei um die sogenannte Küstendouglasie (*Pseudotsuga menziesii* var. *menziesii*) und die Inlandsdouglasie (*Pseudotsuga menziesii* var. *glauca*) (Eckenwalder 2009). Das Verbreitungsgebiet der Küstendouglasie erstreckt sich 2200 Kilometer entlang der Küste von Zentral-Britisch-Kolumbien bis ins zentrale Kalifornien. Das Verbreitungsgebiet der Inlandsdouglasie ist noch ausgedehnter. Über eine Strecke von 4500 Kilometern reicht es von Mexiko bis nach Zentral-Britisch-Kolumbien, wo es sich mit jenem der Küstendouglasie überschneidet und eine Hybridisierungszone bildet (Eckenwalder 2009; Eckert et al. 2009; Gugger et al. 2010; Wei et al. 2011).

Die beiden Varietäten zeigen zudem starke Unterschiede in ihrem Wuchsverhalten. Die Küstendouglasie hat ein besseres Höhen- und Durchmesserwachstum und ist langlebiger als die Inlandsdouglasie. Im Gegensatz dazu ist die Inlandsdouglasie schattentoleranter und kälteresistenter. Zudem bildet sie eine ausgeprägtere Pfahlwurzel aus, was sie vermutlich auch sturmresistenter macht. In feuchteren Gebieten wird die Inlandsdouglasie jedoch bevorzugt vom Pilz *Rhabdocline pseudotsugae* befallen (Hermann & Lavender 1990). Innerhalb dieses ausgedehnten Verbreitungsgebietes finden sich auch unterschiedliche Standortsbedingungen, die verschiedene Anpassungen und Eigenschaften innerhalb der Varietät bewirken. So zeigen beispielsweise nördliche Herkünfte der Küstendouglasie eine höhere Produktivität als südliche, im Gegenzug dazu aber eine geringere Trockentoleranz (Eilmann et al. 2013). Eine große Rolle für die mitteleuropäische Forstwirtschaft spielt aufgrund der heterogenen Standortsbedingungen die Frostempfindlichkeit von Baumarten. So konnte St. Clair (2006) einen starken Zusammenhang zwischen der Temperatur in der kalten Jahreszeit und der Frühfrostempfindlichkeit von Herkunftsgebieten der Küstendouglasie in Oregon und Washington feststellen. Ebenfalls weisen Herkünfte östlich des Kaskadenhauptkamms eine höhere Frosttoleranz auf als westlich davon (Eckert et al 2009).

Diese genannten Unterschiede zwischen und innerhalb der Varietät machen die Herkunftsfrage für die Douglasie essentiell, wenn man erfolgreich Waldbau betreiben will. Deshalb ist diese Frage auch für etablierte europäische Bestände wichtig, da diese natürlich und künstlich weiterverjüngt werden.

Die Geschichte der Douglasie in Europa begann mit ihrer Einführung im 19. Jahrhundert. Damals wurde allerdings die Herkunftsfrage vernachlässigt. Aufforstungen vor den 1980er Jahren sind deshalb meist unbekannter Herkunft (Bastien et al 2013). Die Hauptgründe für

die Forcierung des Douglasien-Anbaus dürften jedoch schon damals Eindrücke von ihren hervorragenden Wuchseigenschaften gewesen sein, was frühe Forschungsergebnisse belegen (Schwappach 1911; Kanzow 1937). Spätere Misserfolge aufgrund der Verwendung von Saatgut aus unterschiedlichen Herkunftsgebieten führte schließlich zu einem Bewusstsein von gezielten Herkunftsversuchen und mündete 1965 in einem von der IUFRO europaweit angelegten Douglasien-Herkunftsversuch (Kleinschmit & Bastien 1992). Die Ergebnisse dieser Versuche empfehlen beispielsweise für den mitteleuropäischen Raum die Küstendouglasie vornehmlich aus den Staaten Washington und Oregon (z.B. Ruetz 1981; Konnert & Ruetz 2006; Weißenbacher 2008). Die Inlandsdouglasie hingegen wird nur in manchen europäischen Ländern mit ausgeprägt kontinentalem Klima empfohlen (Holubčík 1974, 1980; Martinsson 1990; Tigerstedt 1990).

Wie wichtig die Frage der Herkunft ist, zeigt ein seit dem Jahre 1912 laufender Herkunftsversuch in Kaiserslautern. Darin wurden Küstenherkünfte mit Herkünften aus dem Inland und der Hybridisierungszone zwischen Küsten- und Inlandsdouglasie hinsichtlich ihrer Wuchsleistungen miteinander verglichen. Dabei erbrachte die Küstenherkunft Snaquolmie eine Gesamtwuchsleistung von 1958 m³ je Hektar während die beste Nicht-Küstenherkunft in der Hybridisierungszone lediglich 847 m³ je Hektar erbrachte. Die Fichte erbrachte im Vergleich eine Leistung von 1398 m³ je Hektar und erzielte somit höhere Werte als Nicht-Küstenherkünfte, aber niedrigere als die Küstenherkunft (Stimm & Dong 2001). Würde man zwei unterschiedliche Bestände dieser Herkünfte zum Zeitpunkt der Umtriebszeit betrachten und einen durchschnittlichen erntekostenfreien Erlös von 50 € je m³ ansetzen, ergäben sich Unterschiede beim Abtriebserlös von ca. € 34.000,00. Dies führte schließlich in Deutschland dazu, dass Herkünfte aus der Hybridisierungszone oder der Inlandsvarietät nicht mehr als Saatguterntebestände oder für die Weitervermehrung empfohlen werden (Konnert & Ruetz 2006). Auch innerhalb der Küstenherkunft können die Wuchsleistungen stark variieren. So zeigte Weißenbacher (2008), dass in Österreich die Unterschiede zwischen der besten und schlechtesten Küstenherkunft mehr als 30 % betragen.

Diese Forschungsaktivitäten unterstreichen die enorme Bedeutung der Herkunftsfrage bei der forstlichen Verwendung der Douglasie. Da die Herkunft von Beständen, die vor der Publikation der Herkunftsempfehlungen begründet worden sind, meistens nicht bekannt ist, haben dahingehende Herkunftsforschungen eine große Bedeutung. Viele dieser Bestände zeigen herausragende Wuchsleistungen, welche schließlich auch für die Angepasstheit an die jeweiligen Standortsverhältnisse sprechen. Sie werden darum nicht nur natürlich weitervermehrt, sondern auch oftmals als Hauptbezugsquellen für europäisches Douglasien-Saatgut verwendet (z.B. Kohl & Nather 1991; Konnert 2009; Rau 2009). Deshalb braucht es umso mehr fundierte wissenschaftliche Ergebnisse über die Herkunft dieser Bestände, welche allerdings nur mehr forstgenetisch möglich sind. Ein Manko der Herkunftsempfehlungen, die auf dem IUFRO-Versuch basieren, ist, dass sie von relativ jungen Beständen von ca. einer halben Umtriebszeit stammen. Die phänotypisch herausragenden Altbestände repräsentieren oftmals zumindest eine Umtriebszeit. Zur

Validierung dieser Herkunftsempfehlungen ist es also notwendig, auch diese Altbestände hinsichtlich ihrer Provenienz zu untersuchen.

Da die Douglasie ebenso als eine Baumart angesehen wird, die dabei helfen kann, die europäische Forstwirtschaft an die sich veränderten zukünftigen klimatischen Bedingungen anzupassen (IPCC 2007; Eilmann et al. 2013), leistet die Beantwortung der Herkunft dieser Altbestände einen wertvollen Beitrag zur aktuellen Forschung. Zudem ist die Douglasie jetzt schon die wichtigste und häufigste nichtheimische Baumart in den europäischen Wäldern (San-Miguel-Ayanz et al. 2016). Sie hat mit 750.000 Hektar einen Anteil von 0,66 % in den Wäldern Europas, wobei 80 % dieser Flächen in Frankreich, Deutschland und dem Vereinigten Königreich stocken (Bastien et al. 2013). In Österreich ist ihr Anteil mit 0,2% der Waldfläche und knapp 1000 Hektar Fläche (Bastien et al. 2013) noch gering, aber durch verstärkte Aufforstungen in den letzten Jahren im Steigen begriffen. Neben den bereits erwähnten Wuchseigenschaften und der Trockenheitstoleranz sind auch die hervorragenden Holzeigenschaften für die aktuelle und zukünftige Bedeutung mitverantwortlich. Die Douglasie ist nicht nur als Konstruktionsholz oder für die Verwendung im Außenbereich geeignet, sondern auch als Furnier- und Faserholz (San-Miguel-Ayanz et al. 2016).

Diese Arbeit verfolgt die Zielsetzung, die Herkunft von mitteleuropäischen Douglasien-Altbeständen unbekannter Provenienz zu erforschen und so einen Beitrag für eine optimierte forstliche Bewirtschaftung dieser Baumart zu leisten. Die Beantwortung dieser Frage ist in Ermangelung dementsprechender Aufzeichnungen nur anhand forstgenetischer Methoden möglich. Die genetische Zuordnung zu einem bestimmten geographischen Gebiet gelingt umso besser, je höher die Anzahl der Loci, die Anzahl der Allele, die genetische Differenzierung und die Probengröße der untersuchten Populationen ist (Cornuet et al. 1999). Allerdings sprechen Bernatchez & Duchesne (2000) davon, dass 6 – 10 Loci mit einer mittleren Allel-Diversität ausreichend für eine erfolgreiche Zuordnung sind. In der vorliegenden Arbeit wurden für die Herkunftsanalyse 13 Mikrosatellitenloci verwendet (Slovov et al. 2004). Dieser kodominante nukleare Marker zeichnet sich durch einen hohen Polymorphismus aus und eignet sich hervorragend für Zuordnungsuntersuchungen (Estoup et al. 2003). Außerdem wurde er noch nie für eine Analyse der genetischen Differenzierung über das gesamte natürliche Verbreitungsgebiet der Douglasie und der Herkunftsanalyse über die Varietätenzugehörigkeit hinaus verwendet, weshalb mit Beantwortung der Zielsetzung sich auch komplett neue Ergebnisse in der Douglasien-Forschung ergeben und so den Grundstein für weitere Forschung liefern.

2 Zielsetzung und Arbeitsschritte

Die Anfänge der Douglasie in Europa gehen auf das 19. Jahrhundert zurück. Zahlreiche Bestände wurden aus Saatgut unbekannten Ursprungs begründet, da es ja schließlich für Herkunftsempfehlungen keine Ergebnisse aus Versuchsflächen gab und somit jede wissenschaftliche Basis fehlte. Doch die Herkunft ist für einen erfolgreichen Waldbau essentiell. Viele dieser Bestände existieren heute noch in der ersten oder einer späteren Generation bzw. wurden auch weitervermehrt. Gerade diese Bestände sind es, die im Fokus dieser Arbeit stehen. Schließlich haben sie gezeigt, dass sie durch ihre lange Überdauerung von zumindest einer Umtriebszeit und ihres Phänotyps hervorragend an die vorherrschenden standörtlichen Verhältnisse angepasst sind. Dies macht sie aus waldbaulicher Sicht so interessant und wichtig, gilt es doch auf die prognostizierten Klimaänderungen auch forstlich rechtzeitig zu reagieren.

Die vorliegende Arbeit verfolgt deshalb das Ziel, europäische Douglasien-Altbestände unbekannter Herkunft einem Herkunftsgebiet zuzuordnen. Damit dies möglich ist, muss zunächst eine flächendeckende genetische Analyse von möglichst vielen Populationen aus dem gesamten natürlichen Verbreitungsgebiet der Douglasie in Nordamerika durchgeführt werden. Ziel dieser genetischen Analysen ist es, schließlich die Differenzierung innerhalb des Verbreitungsgebietes festzustellen. Nur wenn es innerhalb dieser Gesamtpopulation einen gewissen Grad an Differenzierung gibt, ist es möglich, eine folgende Zuordnung durchzuführen, denn Differenzierung könnte man mit einem Festmachen von Unterscheidungsmerkmalen gleichsetzen. Bei den Merkmalen, die eine Differenzierung innerhalb einer Population beschreiben, handelt es sich um das Vorhandensein gewisser Allele und deren Häufigkeitsverteilung. Ist dieser Schritt getan, wird anhand statistischer Methoden, die in verschiedenen Software-Lösungen zur genetischen Zuordnung integriert sind, je nach Grad der Differenzierung, eine großräumigere oder kleinräumigere Zuordnung von Beständen unbekannter Herkunft getroffen.

Eine allgemeine Übersicht der hier geschilderten Arbeitsschritte ist in Abbildung 1 dargestellt. Sie belegt, dass zur Zielerreichung zwei getrennte Datensätze bestehend aus Referenzmaterial (Datensatz I) und Vergleichsmaterial (Datensatz II) notwendig sind. Datensatz I wird hinsichtlich seiner genetischen Struktur bzw. auch genetischen Differenzierung analysiert. Die Ergebnisse daraus dienen der Entwicklung und Überprüfung einer Zuordnungsmethode. Anhand dieser Methode kann dann Datensatz II einem Ursprungsgebiet zugeordnet werden.

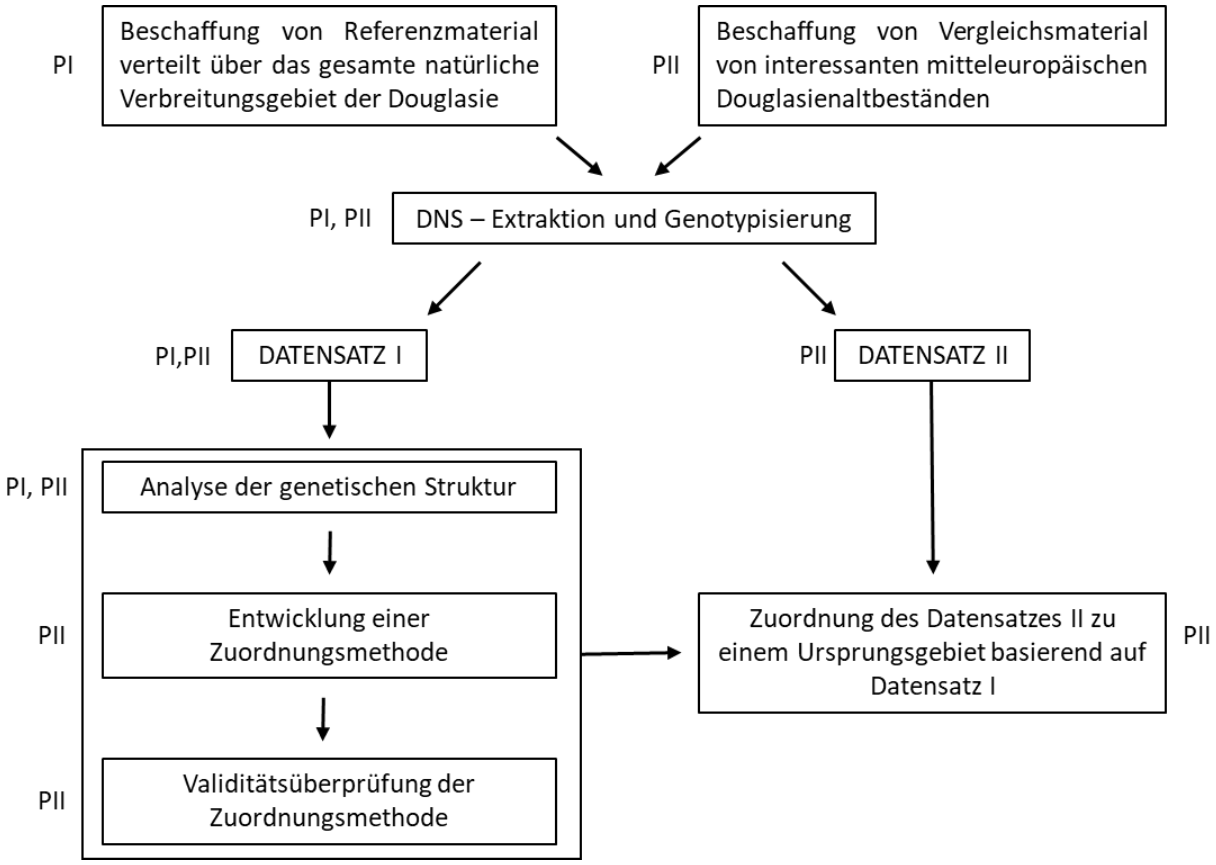


Abbildung 1: *Übersicht der notwendigen Arbeitsschritte zur Zielerreichung. Die Kürzel PI und PII stehen für Publikation I und II. Sie kennzeichnen, welcher der Arbeitsschritte bzw. Datensätze in welcher Publikation behandelt wird (siehe Appendix).*

Die vorliegende Arbeit gliedert sich, basierend auf Abbildung 1, in folgende drei konkrete Schritte:

1. **Analyse der genetischen Struktur** von 38 Referenzpopulationen innerhalb des natürlichen Verbreitungsgebietes der Douglasie als Basis für die genetische Zuordnung von Douglasien-Beständen.

2. **Entwicklung einer Zuordnungsmethode** für die Herkunft von Douglasien-Beständen basierend auf der genetischen Struktur im natürlichen Verbreitungsgebiet der Douglasie und Überprüfung der Validität dieser Methode.

3. **Herkunftsanalyse** von 67 Douglasien-Altbeständen aus Österreich und Deutschland.

3 Daten

Für eine erfolgreiche Zuordnung unbekannter Herkünfte ist es notwendig, eine genetische Differenzierung innerhalb des natürlichen Verbreitungsgebietes festzumachen, um vergleichende Analysen durchführen zu können. Deshalb basiert die vorliegende Arbeit auf zwei Datensätzen. Einer muss aus Populationen bestehen, welche möglichst das gesamte natürliche Verbreitungsgebiet abdecken und ein zweiter Datensatz besteht aus Populationen (bzw. Beständen) unbekannter Herkunft, die man diesem Verbreitungsgebiet zuordnen kann.

Für beide Datensätze wurde dasselbe Verfahren zur DNS-Extraktion und zur Genotypisierung verwendet. Details dazu können den Publikationen I (siehe Anhang I, Kapitel „DNA extraction and genotyping“) und II (siehe Anhang II, Kapitel „DNA extraction and genotyping“) entnommen werden.

3.1 Datensatz I: Populationen aus dem natürlichen Verbreitungsgebiet

Dieser Datensatz umfasst 38 Populationen, welche über das gesamte natürliche Verbreitungsgebiet der Douglasie in Nordamerika verteilt sind (siehe Abb. 1). In den Staaten Oregon und Washington wurde eine höhere Beprobungsdichte gewählt, da die Herkunftsgebiete in diesen beiden Staaten jene sind, die hauptsächlich für Europa empfohlen werden (Breidenstein et al. 1990).

21 der 38 Populationen stammen aus Herkunftsversuchen des Bundesforschungszentrums für Wald (BFW), in Wien, Österreich. Diese Herkunftsversuche stammen aus Saatgut der IUFRO-Beprobung bzw. aus Beprobungen natürlicher Bestände in den USA (vgl. Barner 1973; Chakraborty et al. 2015). Den einzelnen Individuen der Populationen wurden Kambiumproben entnommen.

Die restlichen 17 Populationen wurden aus Saatgut aufgezogen, welches direkt aus den USA und Kanada importiert worden war. Zur Verfügung gestellt wurde es von der „USDA Forest Service-Placerville Nursery“ und dem „British Columbia Forest Service“. Das Saatgut wurde in einem Pflanzbeet ausgesät. Nachdem die Keimlinge 2 bis 3 Monate später eine Höhe von ca. 5 cm erreicht hatten, wurden sie für die DNS-Extraktion geerntet.

Pro Population wurden jeweils 18 bis 22 Individuen beprobt, in Summe ergibt dies eine Stichprobengröße des Datensatzes I von 766 Individuen.

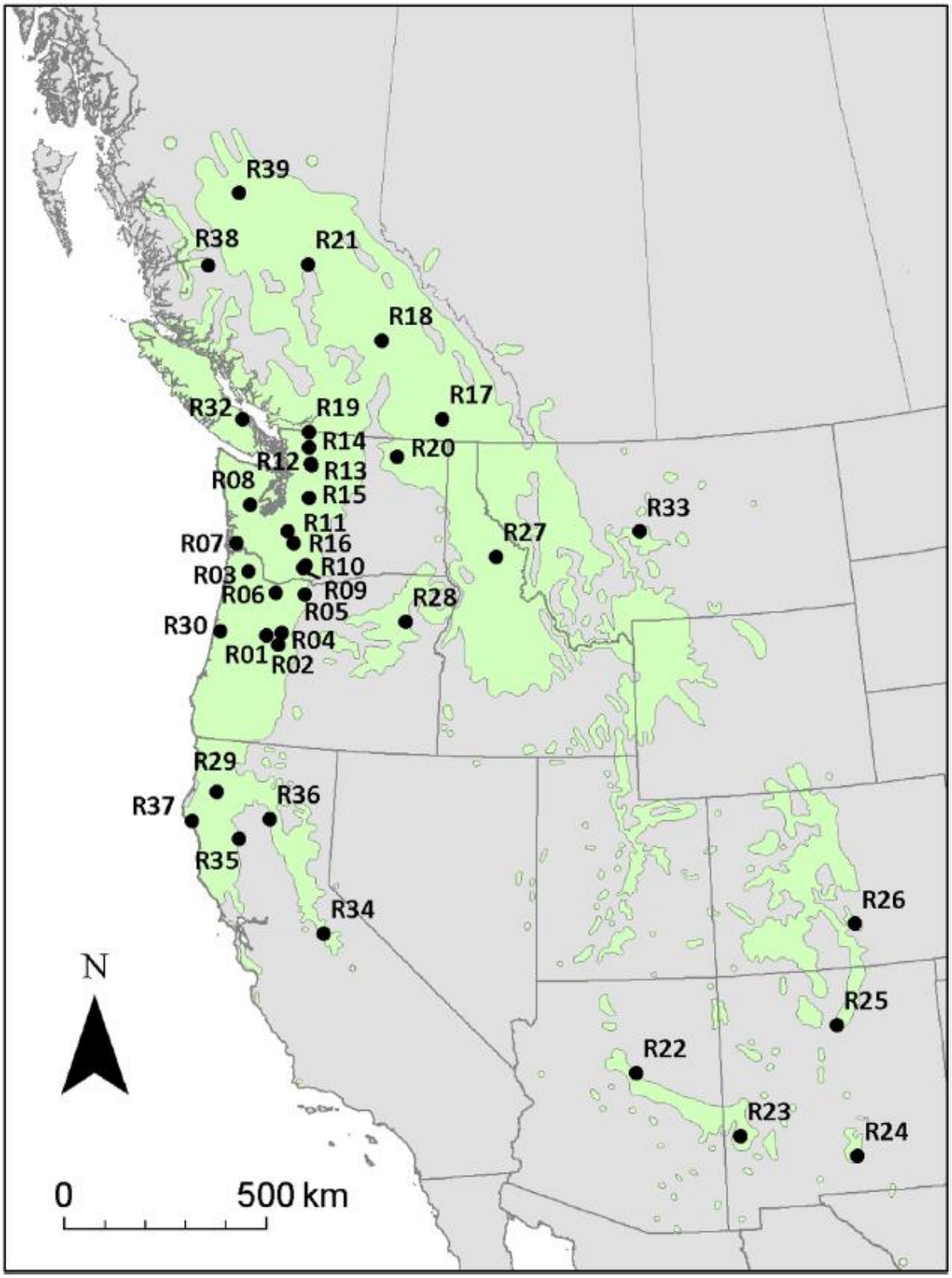


Abbildung 2: *Übersichtskarte der 38 Referenzpopulationen (R01 bis R39, R38 fehlt) innerhalb des natürlichen Verbreitungsgebietes der Douglasie in Nordwest-Amerika*

3.2 Datensatz II: Bestände unbekannter Herkunft

Dieser Datensatz umfasst in Summe 67 deutsche und österreichische Douglasien-Altbestände unbekannter Herkunft (siehe Abbildung 2). Diese Bestände sind aufgrund ihres herausragenden Phänotyps ausgewählt worden bzw. sind sie teilweise auch als zugelassene Douglasien-Saatguterntebestände qualifiziert. Zudem repräsentieren sie oftmals zumindest eine Umtriebszeit. Ein weiteres Kriterium zur Auswahl der Bestände war eine möglichst hohe Standortsheterogenität, um einen möglichen Einfluss des Standorts auf die Herkunft festzustellen. Es wurden innerhalb eines jeden Bestandes von 10 bis 26 zufällig ausgewählten Individuen Kambiumproben entnommen. Dies ergibt in Summe eine Stichprobengröße des Datensatzes II von 1469 Einzelbeprobungen.

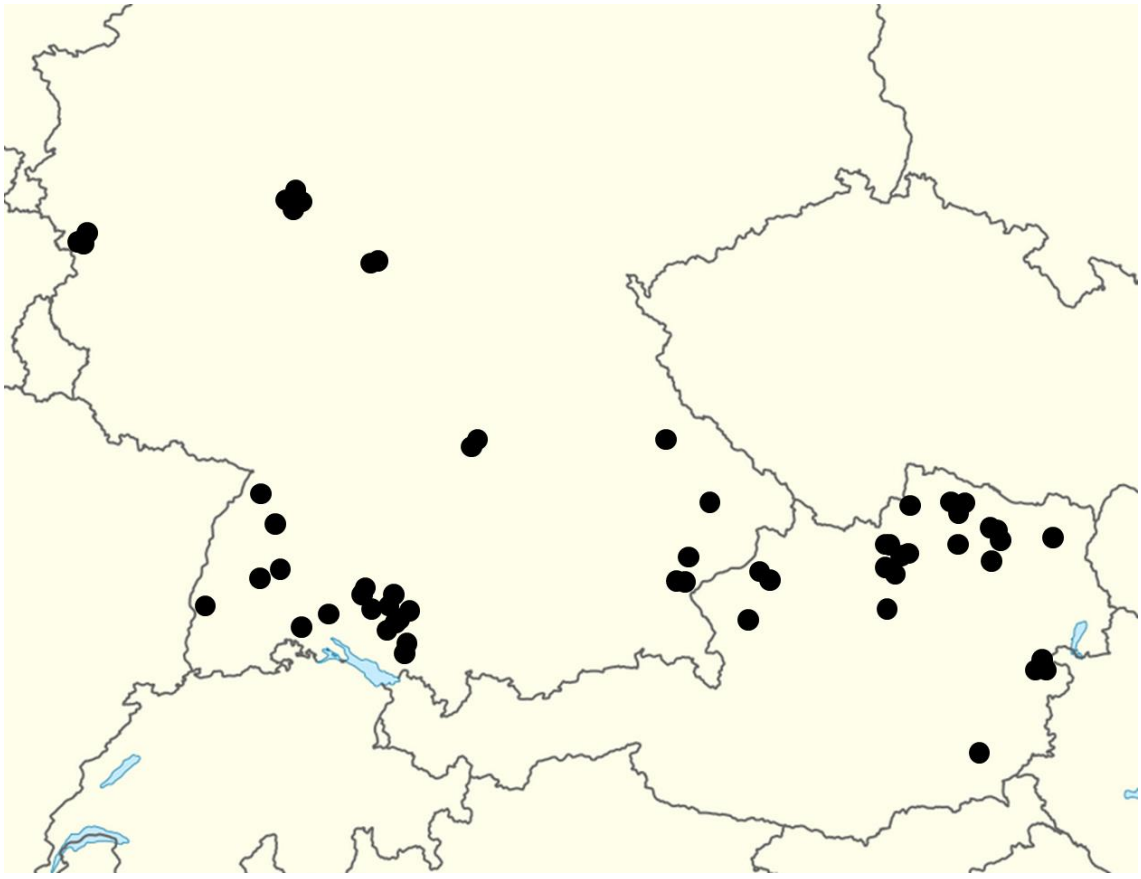


Abbildung 3: *Übersichtskarte der Lage der 67 deutschen und österreichischen Douglasienaltbestände. Einzelne Punkte können auch mehr als einen Bestand repräsentieren.*

Die beiden Datensätze I und II bilden die Basis für die weiteren Analysen in den jeweiligen Arbeitsschritten (siehe Abbildung 1). Die Verortung der Datensätze im Forschungsdesign wird in Tabelle 1 dargestellt.

Parameter		Software	Datensatz		Arbeitsschritt			Publikation	
Kürzel	Beschreibung		I	II	1	2	3	I	II
Parameter zur Analyse der genetischen Diversität									
N_a	Allelanzahl	GenAlEx v. 6.5	x		x			x	
A	durchschn. Allelanzahl	GenAlEx v. 6.5	x		x			x	
N_e	effektive Allelanzahl	GenAlEx v. 6.5	x		x			x	
H_O	beobachtete Heterozygosität	GenAlEx v. 6.5	x		x			x	
H_E	erwartete Heterozygosität	GenAlEx v. 6.5	x		x			x	
F_{IS}	Inzuchtkoeffizient	FSTAT v. 2.9.3.3, GenAlEx v. 6.5	x		x			x	
A_S	Allelischer Reichtum	FSTAT v. 2.9.3.3, ADZE v. 1.0	x		x			x	
HW	Heterozygoten-Defizit	GENEPOP v. 4.1.4	x		x			x	
Parameter zur Analyse der genetischen Differenzierung									
K	Clusteranzahl	STRUCTURE v. 2.3.4, STRUCTURE HARVESTER	x		x			x	x
Q	Cluster-Zugehörigkeitskoeffizient	STRUCTURE v. 2.3.4	x	x	x		x	x	x
Jost`s D	Indikator für genetische Differnzierung	GenAlEx v. 6.5	x		x			x	
F_{ST}	Indikator für genetische Differnzierung	FSTAT v. 2.9.3.3	x		x				x
Parameter zur Analyse der demographischen Geschichte									
t	Abspaltungszeitpunkt	DIYABC v. 2.0	x		x			x	
$N_{1,2,a}$	effektive Größe der jeweiligen Population	DIYABC v. 2.0	x		x			x	
Parameter zur Analyse der Hybridisierung									
IH	intervarietale Heterozygosität	HYBRIDLAB v. 1.1	x		x			x	
HI	Maximum-Liklihood Hybridiserungsindex	HYBRIDLAB v. 1.1	x		x			x	
Parameter zur Analyse der Herkunft									
CA	korrekt zugeordnete Individuen	GENECLASS2 v. 2.0	x			x			x
QI	Qualitätsindex	GENECLASS2 v. 2.0	x			x			x
$score_{i,l}$	Zuordnungstreffer	GENECLASS2 v. 2.0	x	x			x		x
Anzahl der Individuen im jeweiligen Datensatz			**766**	**1469**					

Tabelle 1: *Übersicht der ermittelten Parameter je Datensatz, Arbeitsschritt (siehe Kapitel 2) und Publikation (siehe Appendix).* → *siehe Kommentar auf voriger Seite*

4 Vorgehensweise und Methodik

4.1 Analysekonzept

Der generelle Ablauf und die Gliederung der Arbeit sind in Abbildung 3 dargestellt. Darin wird visualisiert, wie die definierten Arbeitsschritte (siehe Kapitel 2) zusammenhängen und gemeinsam in die Zielsetzung münden. Die jeweiligen berechneten Parameter zu den Arbeitsschritten, Datensätzen und Publikationen sind in Tabelle 1 dargestellt.

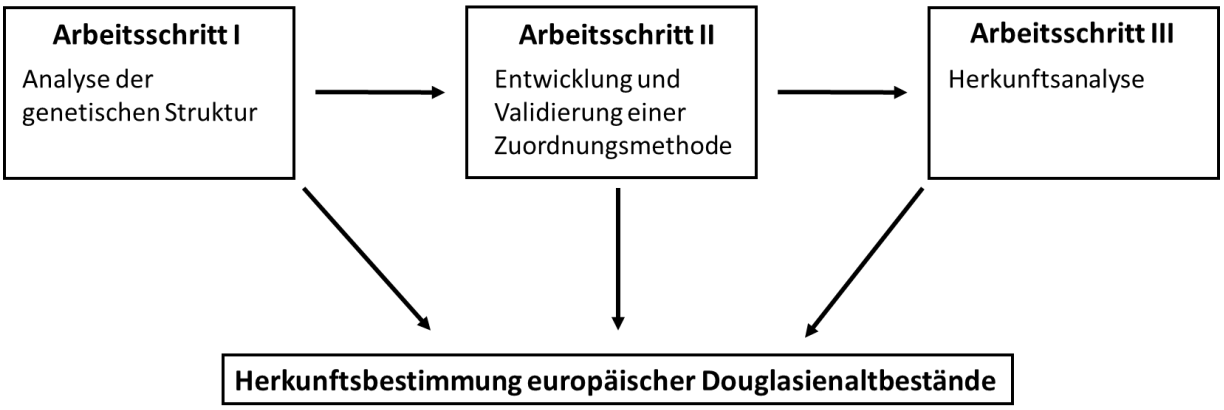


Abbildung 4: *Arbeitsablaufdiagramm zur Zielerreichung*

Im ersten Arbeitsschritt wird die genetische Struktur der 38 Douglasien-Referenzbestände aus Datensatz I analysiert. Wie in Tabelle 1 ersichtlich, beschreibt die genetische Struktur grundsätzlich die Differenzierung innerhalb des natürlichen Verbreitungsgebietes der Douglasie. Zusätzliche Analysen über die Phylogeographie und Diversität liefern weitere Ergebnisse zur Begründung der Struktur. Differenzierung entsteht durch Selektion, Drift und Mutation, wobei ein eingeschränkter Genaustausch zwischen Populationen vorliegen muss. Die Phylogeographie beschreibt den Verlauf und die räumliche Lage dieses Prozesses wie die nacheiszeitliche Ausbreitung aus Refugialgebieten. Refugialgebiete zeichnen sich durch eine erhöhte genetische Diversität aus, weshalb Datensatz I auch dahingehend analysiert wird. Ebenfalls von Relevanz ist die Frage der Hybridisierung im natürlichen Verbreitungsgebiet. Diese kann nämlich einen Einfluss auf die Zuordnung haben. Näher eingegangen wird in weiterer Folge auf die genetische Struktur in Form von Differenzierung. Details dazu wie auch zur genetischen Diversität, zur Hybridisierung und Phylogeographie finden sich in Publikation I und II.

Anhand der genetischen Struktur kann im zweiten Arbeitsschritt eine Zuordnungsmethode basierend auf Datensatz I entwickelt werden. Die Differenzierung beschreibt das Vorhandensein sogenannter genetischer Cluster innerhalb des natürlichen Verbreitungsgebietes. Diese unterscheiden sich charakteristisch hinsichtlich des

Vorhandenseins und der Verteilung bestimmter Allele. Nur mittels dieser differenzierten genetischen Cluster können Bestände unbekannter Herkunft einem wahrscheinlichen Ursprungsgebiet zugeordnet werden. Die darauf basierende Zuordnungsmethode wird in weiterer Folge auf ihre Validität hin überprüft. Schließlich werden im dritten Schritt 67 österreichische und deutsche alte Douglasien-Bestände (Datensatz II) einem Herkunftsgebiet, basierend auf Datensatz I, zugeordnet. Details dazu finden sich in Publikation II.

4.2 Genetische Struktur

Im ersten Arbeitsschritt wird die genetische Struktur von Datensatz I, welche die Basis für eine Herkunftsbestimmung darstellt, analysiert. Genetische Struktur liegt vor, wenn sich Populationen hinsichtlich ihrer Allele und deren Verteilung unterscheiden. Je stärker diese Unterscheidung ausgeprägt ist, desto größer ist auch die Differenzierung. Diese voneinander differenzierten Populationen werden in weiterer Folge in genetischen Cluster zusammengefasst und also solche benannt. Diese können zusätzlich über Diversitätsparameter und deren Phylogeographie beschrieben werden. Die genetischen Cluster werden mittels der Software STRUCTURE v. 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007) ermittelt. STRUCTURE bedient sich dabei einer Bayesian-Clustering-Methode. Neben der Bayesian-Methode finden zum Clustering auch frequenz- und distanzbasierte Methoden Anwendung. Laut Cornuet et al. 1999 erweisen sich bayesian- und frequenzbasierte Methoden geeigneter zur Zuordnung.

Die Bayesian-Clustering-Methode innerhalb von STRUCTURE ordnet die 766 Individuen von Datensatz I einer vorher unbekannten Anzahl K an genetischen Clustern zu. K stellt also jene Anzahl an Populationen dar, die anhand ihrer charakteristischen Allelhäufigkeit an einem bestimmten Locus unterschieden werden können. Jedes Individuum kann dabei mindestens einem Cluster zugeordnet werden. Aus diesem Grund ist es auch möglich, sogenannte beigemischte Individuen oder Hybride zu identifizieren.

Um den gesuchten K-Wert zu erhalten, ermittelt STRUCTURE anhand der Genotypen der Individuen und einem vorher festgelegten Maximalwert für K und für jedes K die „Estimated Ln Prob of Data“ bzw. ln Pr(X|K). Daraus lässt sich dann anhand der ΔK-Methode von Evanno et al. (2005) der K-Wert der höchsten hierarchischen Stufe ableiten. Dieser so ermittelte K-Wert ist Basis zur Untergliederung des Datensatzes I in genetische Cluster bzw. Subpopulationen und somit Spiegel genetischer Strukturen. Details zur Ermittlung der genetischen Struktur können den Kapiteln „Genetic structure and clustering“ (Publikation I, Appendix 9.1) und „Population structure within the reference populations“ (Publikation II, Appendix 9.2) entnommen werden.

4.3 Zuordnungsmethode

In zweiten Arbeitsschritt wird eine Zuordnungsmethode entwickelt und deren Validitätsüberprüfung durchgeführt. Basis für die Zuordnung sind die genetische Struktur und die Gliederung ihrer Cluster in hierarchische Level. Dabei werden die Bestände aus Datensatz II schrittweise beginnend beim höchsten bis zum niedrigsten Level einem immer kleiner werdenden geographischen Gebiet zugeordnet.

Die Software GENECLASS2 (Piry et al. 2004) analysiert über einen Selbstzuordnungstest die Validität dieser hierarchischen Zuordnungsmethode. Dabei wird mittels der Allelfrequenzen von Datensatz I eine gewisse Anzahl an Individuen simuliert, anhand derer die Wahrscheinlichkeit berechnet wird, mit der der Genotyp des Individuums in einem genetischen Cluster auftreten wird. Der Genotyp des Individuums, das gerade zugeordnet werden soll, wird bei der Simulation ausgelassen („leave-one-out approach“). Die Simulation selbst wird mittels der Monte-Carlo-Resampling-Methode nach Paetkau et al. (2004) durchgeführt. Diese berücksichtigt im Vergleich zu den Methoden von Rannala & Mountain (1997) und Cornuet et al. (1999), welche ebenfalls in GENECLASS2 implementiert sind, residente Individuen sowie eine variable Probengröße stärker (vgl. Piry et al. 2004). Als Ergebnis in diesem Arbeitsschritt gelten ein Wert (CA) der zu den genetischen Clustern korrekt zugeordneten Individuen und ein Qualitätsindex (QI). Dieser ist definiert als der Mittelwert der Zuordnungstreffer jedes Individuums in Bezug auf seine ursprüngliche Herkunftspopulation (Piry et al. 2004). Details können dem Kapitel „Accuracy assessment through self-assigment tests within the inferrred clusters“ aus Publikation II (Appendix 9.2) entnommen werden.

4.4 Herkunftsanalyse

Nach der Clusterung von Datensatz I in Subpopulationen bzw. genetische Cluster und der Validitätsüberprüfung der Zuordnungsmethode zu diesen, wird im dritten Arbeitsschritt der Datensatz II (österreichische und deutsche Bestände unbekannter Herkunft) den definierten genetischen Clustern zugeordnet, um so die Herkunft dieser Bestände zu bestimmen. Dies erfolgt über die Software STRUCTURE v. 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007) und GENECLASS2 (Piry et al. 2004). STRUCTURE wird dazu verwendet, Individuen und Bestände des Datensatzes II einer Varietät zuzuordnen, indem für Datensatz I und II ein K-Wert von 2 gesetzt wird. Das Programm GENECLASS2 hat die Bestände aus Datensatz II anhand einer Likelihood-Funktion den Referenzpopulationen von Datensatz I zugeordnet. GENECLASS2 hat gegenüber STRUCTURE den Vorteil, dass dafür nicht die Annahme getroffen werden muss, dass die wahre Herkunftspopulation beprobt worden ist, da hier nicht Populationen miteinander verglichen werden, sondern diese getrennt voneinander betrachtet werden. In GENECLASS2 werden also also Individuen einer mutmaßlichen Population zugeordnet, wenn sie von einer anderen ausgeschlossen werden können (Manel

et al. 2002). Details zur Herkunftsanalyse können dem Kapitel „Origin assigment of European Douglas-fir“ aus Publikation II (Appendix 9.2) entnommen werden.

5 Analyse und Ergebnisse

5.1 Genetische Struktur

Die Analyse der genetischen Struktur wird auf zwei Ebenen (Ebene I und II) durchgeführt. In der ersten Ebene liegt der Schwerpunkt auf der Phylogeographie der Douglasie innerhalb ihres natürlichen Verbreitungsgebiets. Es wird die Lage möglicher eiszeitlicher Refugien untersucht, welche die Ausgangspunkte für die heutige Untergliederung in Subpopulationen darstellen. In der zweiten Ebene wird auf Basis dieser Ergebnisse die Analyse wiederholt, allerdings mit dem Ziel, eine möglichst hohe Untergliederung von Datensatz I in Subpopulationen bzw. genetischen Clustern zu erreichen. Zur Analyse wird die Software STRUCTURE verwendet (siehe Publikation I und II im Appendix I und II). Dabei werden folgende Einstellungen gewählt:

- „admixture" – Modell,
- korrelierte Allelfrequenzen,
- 50.000 „burn-in" – Replikationen,
- 100.000 MCMC – Iterationen,
- 20 Durchläufe per K.

Während auf Ebene I für K noch ein Wert in der Höhe der beprobten Populationen gewählt worden ist, liegt dieser aufgrund der daraus gewonnen Ergebnisse auf Ebene II nur mehr zwischen 1 und 10. Somit kann die Zeit für die Berechnungen reduziert werden. Weiters wird auf Ebene II die gesamte Berechnung mit der „locprior" – Option (Hubisz et al. 2009) wiederholt. Diese gilt als sensitiver zur Aufzeigung genetischer Struktur und eignet sich somit für die Küstendouglasie, welche innerhalb ihres Verbreitungsgebietes eine geringe genetische Differenzierung aufweist (Li & Adams 1989; Hubisz et al. 2009; Krutovsky et al. 2009). Somit kann auch eine stringentere Untergliederung in genetische Cluster erreicht werden. Der jeweilige K-Wert wird über die ΔK-Methode nach Evanno et al. (2005) mittels STRUCTURE HARVESTER (Earl & von Holdt 2012) bestimmt. Begonnen wird mit dem gesamten Datensatz I. Je nach Ergebnis der K-Wert-Ermittlung wird dieser Datensatz in genetische Cluster gesplittet. Basis dafür ist der in STRUCTURE ermittelte Cluster – Anteilswert Q, welcher für jedes Individuum und jede Population ermittelt worden ist. Auf Basis von Q wird in weiterer Folge eine Zuordnung zum jeweiligen Cluster getroffen und die Analyse innerhalb dieser Cluster wiederholt. Die Analysekriterien und -schritte der beiden Ebenen werden im Folgenden kurz beschrieben. Details sind im Kapitel „Genetic structure and clustering" von Publikation I und im Kapitel „Population structure within the reference populations" aus Publikation II nachlesen.

Ebene I

- Für die Anzahl der K-Cluster wird jener Wert gewählt, welcher sich nach der ΔK-Methode beim höchsten ΔK-Wert ergeben hat.

- Die Populationen werden jener Varietät zugeordnet, bei der ihr Anteilswert Q größer 0.9 gewesen ist.
- Innerhalb der Varietät erfolgt eine Zuordnung der Populationen zu jenen Clustern, bei denen ihr Anteilswert Q ohne die „locprior“-Option größer 0.8 gewesen ist.
- Ergibt sich für eine Population ein Anteilswert Q kleiner 0.8 wird die Analyse mit der „locprior“-Option wiederholt. Werden dabei die Populationen zu den gleichen Clustern mit einem Anteilswert Q größer 0.8 zugeordnet, wird das Ergebnis akzeptiert. ISt der Anteilswert Q jedoch unter 0.8 geblieben, werden die Populationen von der weiteren Analyse als „clustergemischte“ Populationen ausgeschlossen.

Ebene II

- Für K wird der Maximalwert für die Anzahl an K-Clustern nach der ΔK-Methode gewählt, der gleichzeitig auch bei jedem der 20 Durchläufe pro K in STRUCTURE zum gleichen Zuordnungsergebnis führt.
- Führen die beiden „locprior“-Optionen (mit und ohne „locprior“) in STRUCTURE zum gleichen Zuordnungsergebnis, werden die Populationen jenem Cluster zugewiesen, bei dem ihr Anteilswert Q größer 0.6 gewesen ist.
- Populationen mit einem Anteilswert Q kleiner 0.6 werden als „clustergemischte“ Populationen von der weiteren Analyse ausgeschlossen.

Für beide Ebenen galt, dass wenn sich bei Wahl der „locprior“-Option eine Zuordnung von Populationen zu anderen Clustern ergibt, oder keine Aufteilung der Populationen in zumindest zwei Cluster mehr möglich ist, die weitere Analyse für dieses Cluster gestoppt wird. Das höchste hierarchische Level ist erreicht. Ein Beispiel dafür, welcher Wert für K bei den oben genannten Analysekriterien gewählt wird, ist in Abbildung 5 abzulesen.

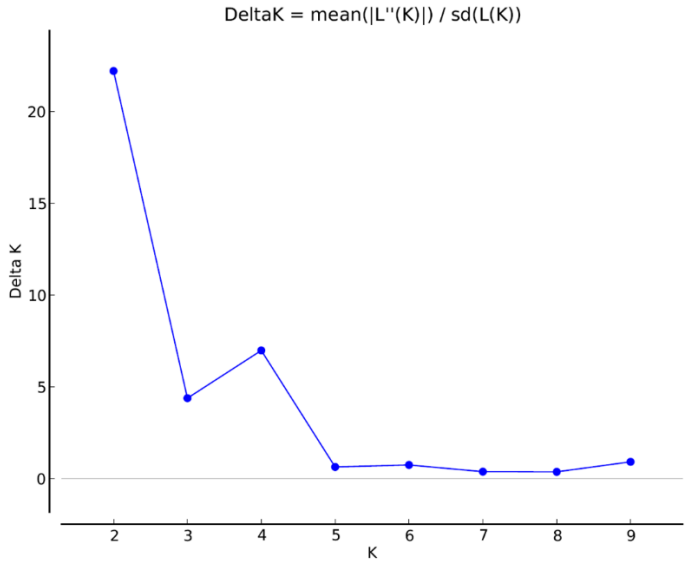


Abbildung 5: *Beispiel zur Wahl von K. Ebene I K = 2; Ebene II K = 4 (Bei gleichem Zuordnungsergebnis der 20 Läufe)*

Beim Vergleich der Analysekriterien wird in der Abbildung belegt, dass bei Ebene I die Grenzwerte für den Anteilswert Q und die Auswahlkriterien für die Anzahl an K-Clustern restriktiver sind. Dies kann mit der unterschiedlichen Schwerpunktsetzung begründet werden. Bei Ebene II wird auch die Zuordnung von Douglasien-Beständen unbekannter Herkunft zu einem engeren geographischen Gebiet ermöglicht. Deshalb wird eine Maximierung der Anzahl für die Zuordnung verlässlicher Cluster angestrebt. Im ersten Analyseschritt der Ebene II werden die Populationen des Datensatzes I zu zwei genetischen Clustern, welche ident mit den beiden Varietäten sind, zugeordnet. Im nächsten Schritt können innerhalb dieser beiden Varietäten jeweils wieder zwei und im dritten Schritt letztendlich insgesamt 12 genetische Cluster unterschieden werden. Diese drei Schritte werden als hierarchische Levels 1 bis 3 (HL1-3) bezeichnet, da auch die Zuordnung der unbekannten Bestände aus Datensatz II hierarchisch anhand dieser Schritte erfolgt. Deshalb wird auch ein zusätzliches hierarchisches Level 4 geschaffen, innerhalb dessen die Zuordnung zu den einzelnen zu HL3 gehörenden Referenzpopulationen erfolgt.
Bei Ebene I können letztendlich acht genetische Cluster unterschieden werden. Diese abweichende Anzahl ergibt sich aufgrund der genannten Abweichungen bei den Analysekriterien. Dennoch belegt Abbildung 6, dass es hinsichtlich der geographischen Lage dieser Cluster nur geringe Unterschiede gibt.

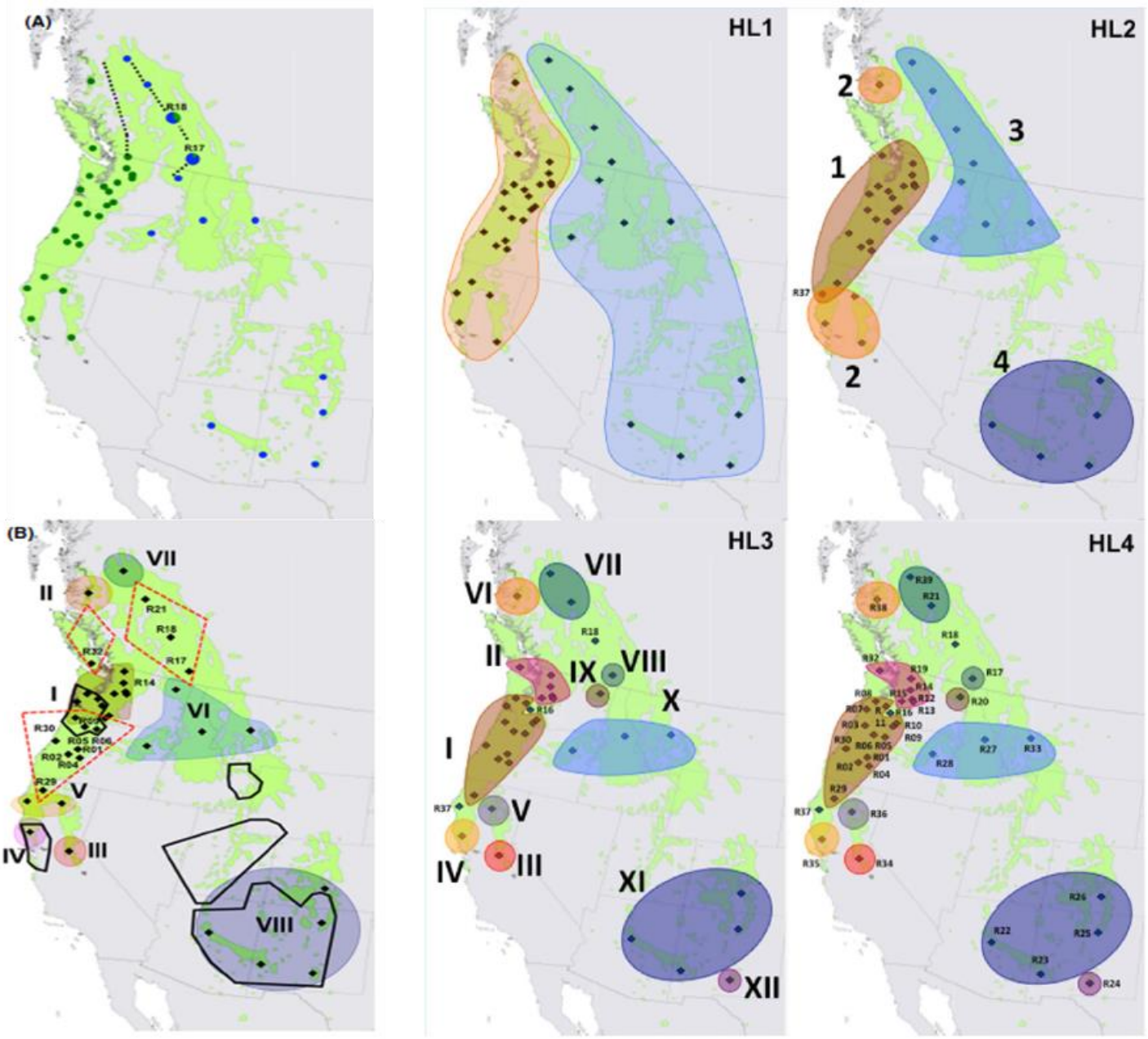


Abbildung 6: *Verbreitungskarten der Douglasienpopulationen aus Datensatz I zu einer Varietät (A, Paper I; HL1 Paper II), oder zu einem genetischen Cluster innerhalb der Varietät (B, Paper I; HL2-4, Paper II) anhand der Analysen mit STRUCTURE. Gepunktete Linien visualisieren die Grenzen der vermuteten Hybridzonen zwischen den Varietäten (von Rudloff 1973; Li & Adams 1989; Gugger et al. 2010; Wei et al. 2011) und schwarze durchgezogene Linien kennzeichnen glaziale Refugialgebiete basierend auf fossilen Daten von Gugger et al. (2010). Rote Linien (B, Paper I) umranden bzw. beschriftete Populationen (HL2 und HL3, Paper II) markieren clustergemischte Populationen*

5.2 Zuordnungsmethode

Als Basis für die Zuordnung dienen die definierten hierarchischen Level (siehe Abbildung 6). Die Zuordnungsmethode ist so konzipiert, dass ein Bestand des Datensatzes II in vier Schritten einem Herkunftsgebiet zugeordnet wird. Diese vier Schritte sind gleichzusetzen mit den vier hierarchischen Levels (siehe Abbildung 6 HL1 bis HL4). Somit wirde Schritt für Schritt das Herkunftsgebiet immer näher eingeschränkt, beginnend von der Ebene der Varietät bis hin zu einer der 37 Referenzpopulationen aus Datensatz I.

Um die Zuordnung zu den genetischen Clustern innerhalb der verschiedenen hierarchischen Level zu überprüfen, wird ein sogenannter Selbstzuordnungstest mit der Software GENECLASS2 (Piry et al. 2004) mit dem Berechnungskriterium nach Rannala & Mountain (1997) und der Simulationsalgorithmus von Peatkau et al. (2004) mit einer Stichprobenwiederholung von 10.000 simulierten Individuen und einem Type I Fehler (Alpha) von 0.01 (Cornuet et al. 1999; Paetkau et al. 2004) angewandt. Jedes Individuum des Datensatzes I, welches im jeweiligen hierarchischen Level (HL) einem genetischen Cluster zugeordnet worden ist, wird so dem genetischen Cluster erneut zugeordnet. Allerdings wird das jeweilige Individuum selbst während der Analyse aus dem genetische Cluster entfernt (=„leave-one-out approach“). Das Ergebnis stellt einen Wert dar, der angibt, wieviel Prozent der Individuen wieder ihrem jeweiligen Cluster zugeordnet werden konnten. Dieser Wert wirde mit CA (= „correctly assigned individuals“) abgekürzt. Eine weitere ermittelte Kennzahl ist der in Kapitel 4.2 erklärte Qualitätsindex (QI). Über diese beiden Parameter können Rückschlüsse über die Validität der Zuordnungsmethode gezogen werden.

Die Ergebnisse des Selbstzuordnungstests auf Ebene der hierarchischen Levels sind in Abbildung 7 dargestellt, worin belegt wird, dass mit zunehmendem hierarchischem Level die Werte für CA und QI sinken. So werden im HL1 noch 94,4% der Individuen ihrer jeweiligen Varietät korrekt zugeordnet. Auf Ebene der 12 Cluster (HL3) liegt der Wert noch bei 75,3%. Somit kann das Herkunftsgebiet auf Ebene der 12 Cluster mit hoher Wahrscheinlichkeit bestimmt werden. Der niedrigste Wert für CA wird bei der Zuordnung auf der Populationsebene (HL4) innerhalb des Clusters I (HL3) erzielt. Hier sind es 33,1 % der Individuen, die korrekt zugeordnet werden können. Somit liegt auf Populationsebene eine gewisse Unsicherheit bei der Zuordnung vor. Wäre diese hierarchische Gliederung allerdings nicht vorgenommen worden, und hätte man stattdessen den Selbstzuordnungstest für den kompletten Datensatz I vorgenommen, so betrüge der Wert für CA 32,0 % und wäre somit noch niedriger. Ebenfalls wäre damit eine größere Unsicherheit in Bezug auf die Eingrenzung des Herkunftsgebietes damit verbunden.

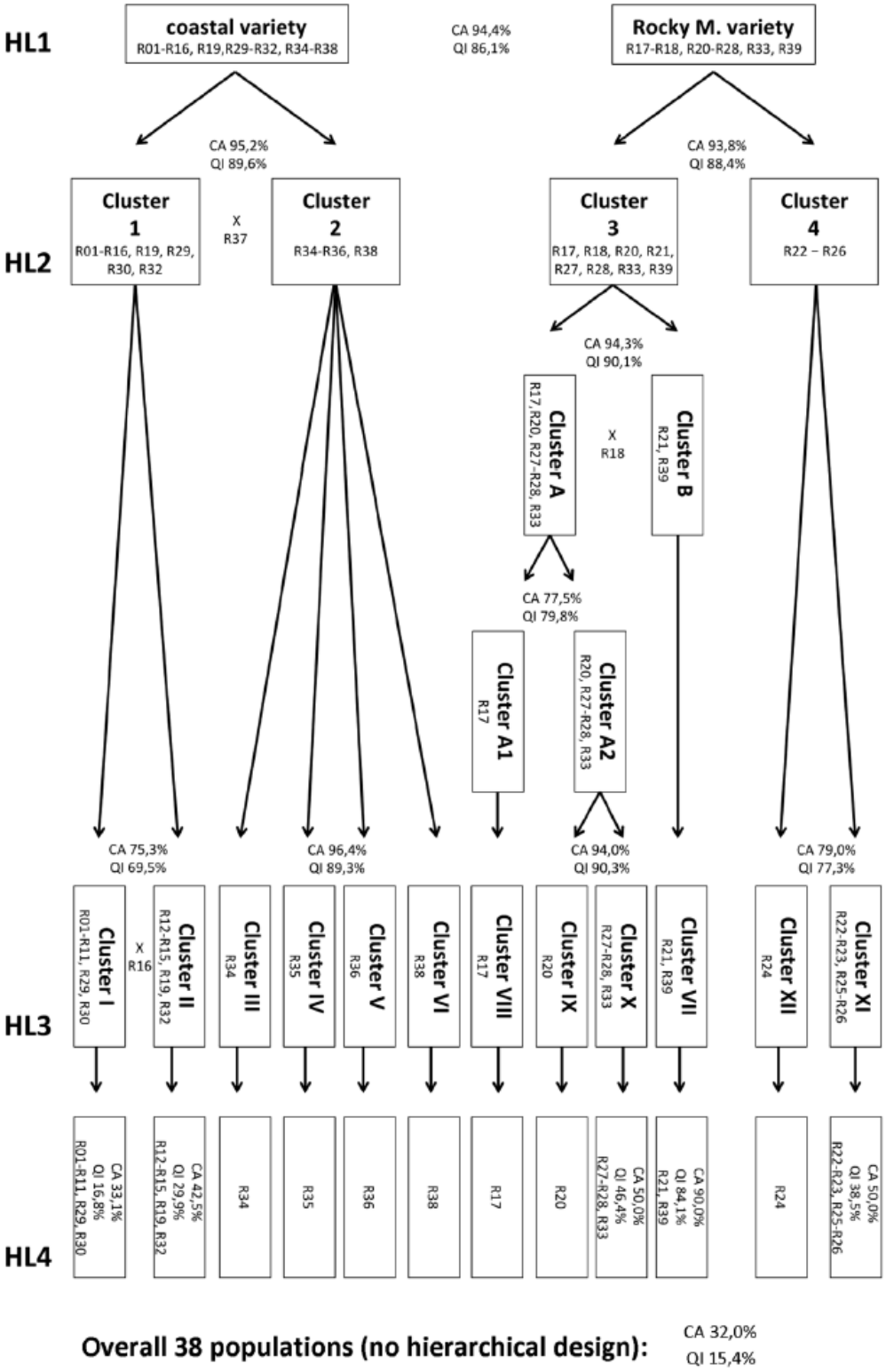


Abbildung 7: *Anordnung der hierarchischen Level (HL1 – HL4) beginnend von den 38 Referenzpopulationen (Datensatz I), welche in die beiden Varietäten aufgegliedert werden, gefolgt von 4 Clustern in HL2 und den finalen 12 Clustern in HL3. Für jeden Zweig der hierarchischen Struktur wird die Anzahl der korrekt zugeordneten Individuen (CA) und der Qualitätsindex (QI) über den Selbstzuordnungstest in GENECLASS2 berechnet. Populationen, welche mit einem X zwischen zwei Clustern markiert sind, sind clustergemischt.*

5.3 Herkunftsbestimmung

Nachdem die Cluster innerhalb ihres hierarchischen Levels anhand des Selbstzuordnungstests auf ihre Validität hin geprüft worden sind, können die 67 Bestände aus Datensatz II einem Herkunftsgebiet zugeordnet werden. Dies erfolgt ebenfalls mit der Software GENECLASS2 (Piry et al. 2004). Diese Bestände werden als „groups of individuals“ anhand der Multilokus-Bayesian-Methode von Rannala & Mountain (1997) mit einem Zuordnungsgrenzwert von p=0.05 hierarchisch zunächst einer Varietät (HL1), danach genetischen Clustern innerhalb dieser Varietät (HL2-3) und abschließend einer Population aus Datensatz I innerhalb dieser Cluster zugeordnet (HL4). Das Zuordnungsschema ist ebenfalls in Abbildung 7 dargestellt. Vor der Zuordnung der Bestände sind diese allerdings noch von Individuen der jeweiligen anderen Varietät bereinigt worden. Individuen mit einem Q – Wert (siehe Kapitel 5.1) größer 0.9 zu einer der beiden Varietäten werden separiert und extra einer Zuordnung unterzogen. Es kann nicht ausgeschlossen werden, dass diese Individuen künstlich in den Bestand eingebracht worden sind. Mit dieser Bereinigungsmaßnahme wird eine Verfälschung des Zuordnungsergebnisses vermieden.

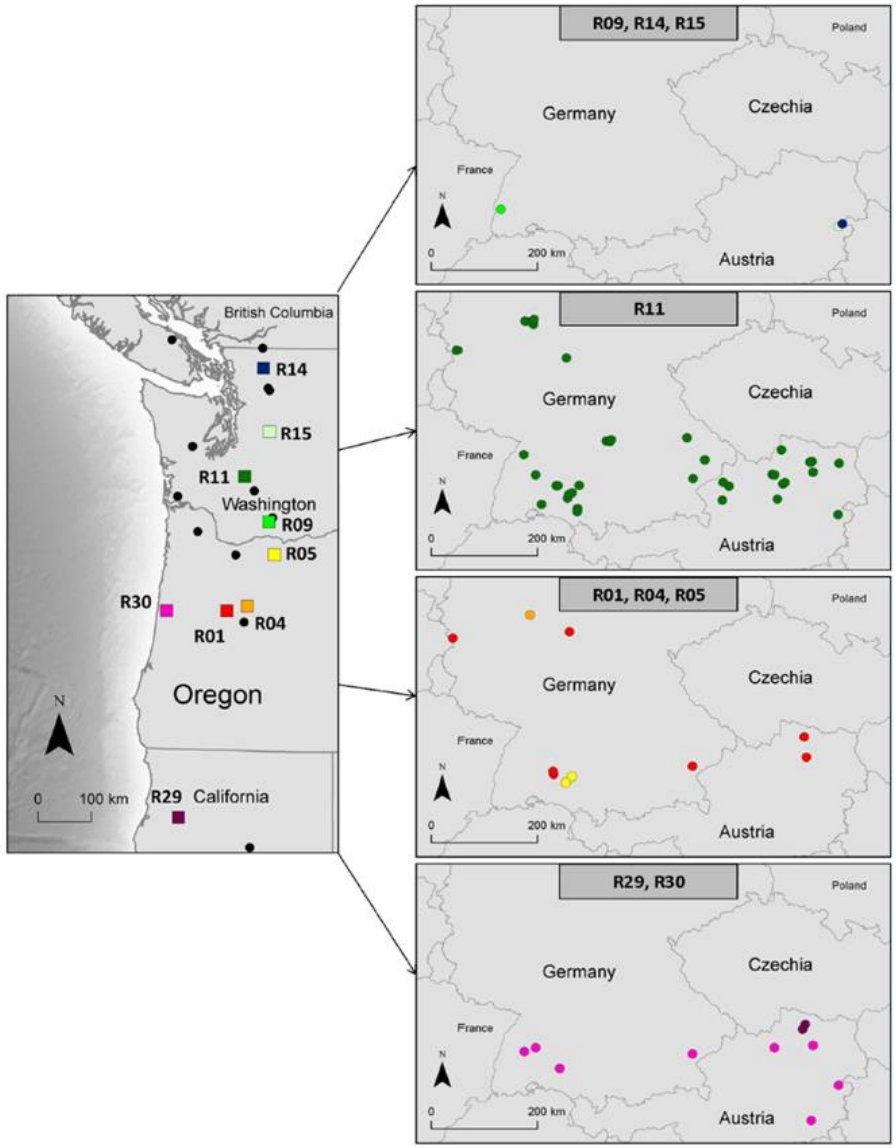
Die schrittweise Herkunftsbestimmung anhand der Zuordnungsmethode ergibt, dass von den 67 Beständen des Datensatzes I 64 Bestände der Küstenvarietät und 3 der Inlandsvarietät zugeordnet werden (siehe Tabelle 2), wobei eine Herkunft daraus einer Region um Sante Fe im US-Bundesstatt New Mexico abstammt.

HL1	HL2	HL3	HL4	N	Name des Ursprungs	Staat	Breite [°]	Länge [°]	Seehöhe [m]
Küstenvarietät	**1**	**I**	**R01**	**7**	Cascadia	US-OR	44,41	122,47	525
			R04	**1**	Marlon Forks	US-OR	44,50	122,00	975
			R05	**2**	Durfur	US-OR	45,40	121,38	975
			R09	**1**	Trout Lake	US-WA	45,97	121,53	675
			R11	**40**	Ashford Elbe	US-WA	46,75	122,13	525
			R29	**2**	Kalifornien (Zone 303)	US-CA	40,85	123,43	100
			R30	**9**	Oregon (Zone 061)	US-OR	44,38	123,88	225
			R14	**1**	Concrete	US-WA	48,65	121,72	475
			R15	**1**	Snoqualmie River	US-WA	47,54	121,55	525
Inlandsvarietät	**3**	**Intermediat von VII und VIII, keine Zurodnung auf**		**2**					
	4	**XII**	**R25**	**1**	Santa Fe N.F.	US-NM	35,75	105,83	2700

Tabelle 2: *Zuordnungsergebnis über GENECLASS2 über die Ebenen HL1 bis HL4. Die Cluster entsprechen der Anordnung in Abbildung 6. N steht für die Anzahl der jeweils zugeordneten Douglasienbestände des Datensatzes II. Ebenfalls angegeben ist der Name des Ursprungs der Referenzpopulation mit dem jeweiligen Staat und den Koordinaten.*

Innerhalb der Küstenvarietät beschränkt sich das Zuordnungsgebiet auf die Bundesstaaten Washington, Oregon und Nordkalifornien. Somit werden innerhalb des Verbreitungsgebietes der Küstenvarietät keine Bestände den Gebieten Südkalifornien oder Britisch-Kolumbien zugeordnet. Im letzten Schritt werden von 43 der 64 Bestände die Westkaskaden im Bundesstatt Washington als Ursprungsgebiet ermittelt (siehe Abbildung 8). Dies entspricht

den Populationen R09 (Trout Lake), R11 (Ashford Elbe), R14 (Concrete) und R15 (Snaquolmie River). Somit stammt ein Großteil der Bestände aus einem relativ kleinen Gebiet, welches sich im Bereich deutscher und österreichischer Herkunftsempfehlungen befindet (Schultze und Raschka 2002, Konnert et al. 2008, Weißenbacher 2008). Die restlichen 21 Bestände haben ihren Ursprung in den Bundesstaaten Oregon und Kalifornien und liegen damit teilweise außerhalb dieser empfohlenen Herkunftsgebiete, wie beispielsweise R29 (Kalifornien Zone 303) und R30 (Oregon Zone 061). Die genauen Details zum Zuordnungsergebnis der 64 Bestände, welche der Küstenvarietät zugeordnet werden, können Abbildung 8 entnommen werden. Diese Ergebnisse sind auch Basis für die Untersuchung des Einflusses des Standorts auf Douglasien-Bestände. Schließlich werden dort 11 Bestände aus Österreich und Deutschland mit einem einheitlichen Herkunftsgebiet (siehe Abbildung 8 rechts) ausgewählt um Einflüsse der Herkunft auf das Wachstum möglichst zu reduzieren. Details dazu können Publikation III entnommen werden.



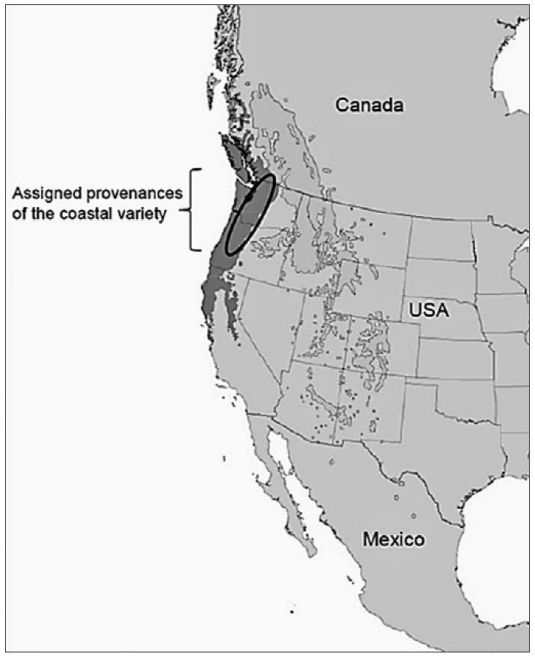


Abbildung 8: *Die Übersichtskarten links und in der Mitte zeigen die Zuordnung der europäischen Bestände zu den Referenzpopulationen in Nordamerika. Die farbigen Quadrate in der linken Karte markieren die unterschiedlichen Referenzpopulationen, in denen eine Zuordnung erfolgt ist, die schwarzen Punkte sind Referenzpopulationen ohne Zuordnung. Die Karte in der Mitte zeigt die Lage der europäischen Bestände, wobei die jeweilige Farbe mit jener der zugeordneten Referenzpopulation korreliert. Die rechte Karte stellt das Herkunftsgebiet der in Paper III untersuchten Bestände dar.*

6 Diskussion

Die vorliegende Arbeit macht es möglich, Douglasien-Bestände unbekannter Herkunft einem Ursprungsgebiet zuzuordnen. Anhand des Vergleichs der Allelfrequenzen der Referenzpopulationen und jener von Beständen unbekannter Herkunft kann eine Zuordnung getroffen werden. Dazu sind voneinander differenzierte Referenzpopulationen quer über das gesamte natürliche Verbreitungsgebiet notwendig. Diese Differenzierung wird ebenfalls aufgezeigt. Die Differenzierung zwischen den Referenzpopulationen kann nur durch genetische Veränderung entstehen. Hauptverantwortlich dafür sind Evolutionsfaktoren, wie zum Beispiel Mutation, Selektion, Drift, Isolation oder Separation.

So haben sich die Küsten- und Inlandsvarietät der Douglasie spätestens in der Mitte des Pleistozäns entwickelt (van Loo et al. 2015, Gugger et al. 2010, Wei et al. 2011, Li & Adams 1989). Innerhalb dieser Varietäten sind während der letzten Eiszeit Refugien entstanden, in denen bedingt durch die Evolutionsfaktoren Differenzierung begünstigt worden ist. Gugger et al. (2010) spricht sich aufgrund von Fossilfunden für 2 eiszeitliche Refugien der Küstendouglasie aus. Die vorliegenden Analysen mit STRUCTURE haben letztendlich fünf genetische Cluster unterschieden, weshalb hier auch von zumindest fünf möglichen Refugien der Douglasie während der letzten Eiszeit ausgegangen wird. Die Analysen auf Ebene II haben lediglich ein Cluster mehr im Bereich von Oregon (siehe Cluster I Publikation II) festgemacht. Dieser Unterschied kann auf die niedrigeren Grenzwerte für Q, die angepassten Auswahlkriterien für K bzw. auf die Stochastizität bei der Bayesian-Clustering-Methode zurückgeführt werden (Gilbert et al. 2012). Bei der Inlandsvarietät werden zunächst drei genetische Cluster unterschieden. Von diesen ist nur eines in Bezug auf das Verbreitungsgebiet ident mit den bei Gugger et al. (2010) aufgezeigten drei Refugialgebieten. Dies kann aber auch auf die geringere Beprobungsdichte bei der Inlandsvarietät mit fehlendem Referenzmaterial aus gewissen Gebieten zurückgeführt werden. Nichtsdestotrotz können bei den Analysen auf Ebene II letztlich sechs genetische Cluster unterschieden werden. Somit stehen insgesamt 12 genetische Cluster für die Zuordnung der mitteleuropäischen Altbestände zur Verfügung.

Doch nicht nur die Anzahl der Cluster ist relevant, auch die Zuordnung von Individuen oder Gruppen von Individuen, wie zum Beispiel eine gewisse Anzahl an beprobten Bäumen aus einem Douglasien-Bestand, die diesen zugeordnet werden kann, ist entscheidend. In diesem Zusammenhang steigt die Unsicherheit der Zuordnung mit Zunahme des hierarchischen Levels. Während bei HL1 noch 94,4 % der Individuen beim Selbstzuordnungstest mit einem QI von 86,1 % korrekt zugeordnet werden können, wird bei HL3 zwischen den Clustern I und II ein Wert für CA von 75,3 % mit einem QI von 69,5 % erreicht. Dies bedeutet, dass die Wahrscheinlichkeit einem dieser beiden Cluster korrekt zugeordnet zu werden bei knapp über 75% liegt. Die Grenze zwischen diesen beiden Clustern liegt im Zentrum des Bundesstaates Washington. Eine Unterscheidung zwischen diesen ist für die Herkunftsbestimmung äußerst wichtig, da gerade aus dem Bereich Washington und Oregon

die empfohlenen Herkunftsgebiete für den mitteleuropäischen Raum stammen (Konnert et al. 2008; Weißenbacher 2008).

Dies wird dadurch bekräftigt, dass von den 67 europäischen Douglasien-Beständen 64 den Clustern I und II zugeordnet werden können, davon wiederum 62 Bestände Cluster I. Innerhalb der Cluster I und II können zwischen den Herkünften bereits Unterschiede in der relativen Wuchsleistung von über 20 % auftreten (Weißenbacher 2008). Interessant ist deshalb auch, dass 40 Bestände einer einzigen Referenzpopulation zugeordnet werden, Population R11 (Ashford/Elbe, Washington). Folgt man den Empfehlungen von Schultze & Raschka (2002), ist dies positiv zu werten, da sie die Herkunft Ashford (Washington) ebenfalls für den Sommerwarmen Osten Österreichs empfehlen. Auch Kleinschmit et al. (1991) sprechen sich nach Auswertung des 20jährigen IUFRO-Provenienzversuchs für Herkünfte westlich des Kaskadenhauptkammes in Washington, auf der Olympic – Halbinsel und dem nördlichen Oregon zur Verwendung in Deutschland aus. Diese Herkunftsempfehlungen basieren auf den Ergebnissen des IUFRO-Provenienzversuchs (Barner 1973), in welchem die untersuchten Herkünfte durch ihre Wuchseigenschaften überzeugt haben. Mit Wuchseigenschaften sind nicht nur Durchmesser-, Höhen- oder Volumsleistungen zu verstehen, sondern auch die Anfälligkeit gegenüber biotischen (z.B. Rostige Douglasien-Schütte) und abiotischen (z.B. Frost) Schadereignissen sowie qualitätsbestimmende Merkmale. Dies wirkt sich direkt auch in der Wertleistung aus, was Kleinschmit (2002) ökonomisch für die Douglasie nachweisen konnte. Er verglich die 25 % besten und schlechtesten Douglasien-Herkünfte eines Herkunftsversuches hinsichtlich ihrer Wertleistung. Dabei lag die Wertleistung der besten und schlechtesten Herkünfte um 15 bis 20 % über bzw. unter dem Versuchsmittelwert. Somit hatten die schlechtesten Herkünfte gegenüber den besten, bezogen auf einen Produktionszeitraum von 100 Jahren, einen um 26.000 €/ha geringeren erntekostenfreien Abtriebserlös.

Was sind mögliche Gründe dafür, dass die europäischen Altbestände zu einem Großteil aus Gebieten stammen, die später durch Versuche als empfohlene Herkunftsgebiete bestätigt worden sind? Zwei Erklärungsansätze scheinen plausibel. Wenn Bestände geeigneter und ungeeigneter Herkunft angepflanzt werden, bleiben durch Selektion nur die besser geeigneten über, oder es sind, bestärkt durch frühe Forschungsergebnisse (Schwappach 1911, Kanzow 1937), Bestände aus geeignetem Herkunftsmaterial bevorzugt etabliert worden. Dennoch können Bestände der Küstendouglasie auch außerhalb dieser empfohlenen Herkunftsgebiete zugeordnet werden. So werden beispielsweise neun Bestände einer Referenzpopulation (R30) aus der Samenzone 061 (Oregon) und zwei Bestände einer Referenzpopulation (R29) aus dem nördlichen Kalifornien zugewiesen. Somit stellt sich die Frage, ob es auch außerhalb der empfohlenen Herkunftsgebiete mögliche geeignete Provenienzen gibt? Allerdings erfolgt keine Untersuchung nach der Wuchsleistung und so kann man nicht beurteilen, ob die zugeordneten Bestände außerhalb der empfohlenen Herkunftsgebiete die gleiche Leistung wie innerhalb dieser erbringen. Für den erfolgreichen Anbau einer Baumart sind neben der Herkunft auch noch die Faktoren genetische Diversität und Standort entscheidend. Die Analyseergebnisse dieser Arbeit lassen

nicht darauf schließen, dass bestimmte Herkünfte nur bestimmte Standorte bevorzugen. Dies wird durch die bereits erwähnte Zuordnung von 40 europäischen Beständen zur Referenzpopulation R11 (Ashford/Elbe, Washington) belegt. Bestände dieser Herkunft werden sowohl im Osten Österreichs als auch im südlichen und mittleren Westen Deutschlands gefunden.

In dieser Arbeit wird überdies die genetische Diversität im natürlichen Verbreitungsgebiet untersucht (siehe Publikation I im Anhang I). Diese ist innerhalb der Cluster I und II am höchsten. Diese befinden sich auf dem Gebiet eines eiszeitlichen Refugiums der Douglasie (Gugger et al. 2010) und eine hohe genetische Diversität ist dafür nach dem Refugialkonzept von Hewitt (1996) typisch. Je höher die genetische Diversität desto höher ist auch die Anpassungsfähigkeit. Deshalb ist diese eine wichtige Eigenschaft zur Besiedelung neuer Standorte oder Widerstand gegen äußere Störungen. Aus diesem Grund kommt der genetischen Diversität auch bei einer Klimaänderung große Bedeutung zu. Dass ein Großteil der Bestände einem Gebiet mit erhöhter genetischer Diversität zugeordnet werden kann, spricht auch für die Anpassungsfähigkeit dieser Bestände. Neben der Rolle der Herkunft und genetischen Diversität wir auch die Rolle des Standorts auf das Douglasien-Wachstum untersucht (siehe Publikation III). Alle drei Faktoren spielen für einen erfolgreichen zukünftigen Douglasien-Waldbau eine große Rolle. Aus den genannten Gründen leistet die vorliegende Arbeit einen wichtigen Beitrag zum besseren Verständnis und mehr Information über die europäische Douglasie. Sie unterstützt das Betreiben eines zielgerichteten Waldbaus und ein proaktives forstliches Reagieren auf prognostizierte Klimaänderungen in Zukunft.

7 Schlussfolgerungen und Ausblick

Die vorliegende Arbeit hat eine Zuordnungsmethode entwickelt, die Douglasien-Bestände unbekannter Herkunft einem bestimmten Herkunftsgebiet erfolgreich zuordnen kann. Optimierungspotenzial liegt in einer lückenlosen Erweiterung der Referenzbestände und in einer Erhöhung der Anzahl von Mikrosattelitenloci. Die in dieser Arbeit zugeordneten Bestände stammen zum Großteil aus einem für Mitteleuropa empfohlenen Herkunftsgebiet. Somit eignen sich diese Bestände für die Weitervermehrung und stellen eine Basis für eine „europäische Douglasie“ dar. Bestände mit beigemischten Individuen der Inlandsvarietät sollten allerdings wie in Deutschland (Konnert & Ruetz 2006).) nicht für eine Saatgutgewinnung zugelassen werden. Generell sollte eine Weitervermehrung solcher gemischten Bestände bzw. Bestände aus reiner Inlandsdouglasie überdacht werden, wenn man produktive und stabile Waldbestände erhalten will. Es ist deshalb ratsam, die Herkunftsbestimmung auf möglichst alle phänotypisch herausragenden europäischen Douglasien-Altbestände zu erweitern. Es bleibt dann abzuwarten, ob diese ebenso aus einem relativ einheitlichen Herkunftsgebiet stammen bzw. den nationalen Herkunftsempfehlungen folgen.

Ebenfalls wichtig ist eine zukünftige Analyse der genetischen Diversität der Altbestände. Nur Bestände mit einer hohen genetischen Diversität garantieren ein entsprechendes Anpassungspotential bei sich verändernden bzw. störenden Umweltbedingungen. Da diese Arbeit Teil eines größeres Douglasien-Projekts mit den weiteren Forschungsschwerpunkten Standort, Naturverjüngung und Klimawandel ist, wurde in dessen Rahmen auch untersucht, ob in der Verjüngung ein Diversitätsverlust eintritt. Eckert et al. 2017 wiesen beim Vergleich von Sämlingen europäischer und amerikanischer Douglasien-Saatguterntebestände eine geringere genetische Diversität als bei europäischen Sämlingen nach. Aus diesem Grund wurde die Verwendung von amerikanischem Saatgut empfohlen. Die geringere Diversität der europäischen Bestände liegt vermutlich an der isolierten Lage und der geringen Individuenanzahl.

Was macht man also mit den hier untersuchten Beständen, bei denen man eine geeignete Herkunft festgestellt hat und mit Naturverjüngung arbeiten will? Hier erscheint es in Zukunft wichtig, diese Bestände mit Pflanzen aus dem natürlichen Herkunftsgebiet zu stützen, um die genetische Diversität zu erhöhen. Weiters beschreiben Eberhard & Hasenauer (2018) in ihrer Arbeit, dass Douglasien-Verjüngung sensibel auf Konkurrenz vornehmlich durch die Buche reagiert und so nur ein geringes Potential zur Invasivität vorweist. Deshalb scheint neben der Wahl geeigneter Standorte (siehe Publikation III, Appendix 9.3; Eckhart et al. 2018) eine zielgerichtete Bewirtschaftung der Douglasien-Bestände unerlässlich, wenn man diese Baumart zukünftig forcieren will, um die europäische Forstwirtschaft hinsichtlich des Klimawandels zu adaptieren.

Abschließend lässt sich festhalten, dass die generierten Ergebnisse einen wertvollen Beitrag für die Douglasien-Bewirtschaftung in Europa leisten, da Bestände untersucht worden sind,

welche zumindest eine Umtriebszeit repräsentieren, gute Wuchsleistungen zeigen, sich natürlich verjüngen und zum Teil auch als anerkannte Saatguterntebestände zugelassen sind. Die Douglasie wird unter den prognostizierten Klimaänderungen in Zukunft eine gewichtigere Rolle in den europäischen Wäldern spielen und diese Arbeit hat einen Beitrag dazu geleistet.

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9 Appendix

9.1 Publikation I

Van Loo M*, Hintsteiner W*, Pötzelsberger E, Schüler S, Hasenauer H (2015) Intervarietal and intravarietal genetic structure in Douglas-fir: nuclear SSRs bring novel insights into past population demographic processes, phylogeography, and intervarietal hybridization. Ecology and Evolution 5(9):1802-1817

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Intervarietal and intravarietal genetic structure in Douglas-fir: nuclear SSRs bring novel insights into past population demographic processes, phylogeography, and intervarietal hybridization

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Keywords
Douglas-fir, genetic diversity, genetic structure, intervarietal hybridization, refugia, varieties.

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Funding Information
MT was founded by the Austrian Science Fund (FWF) (Project-ID: P26504) and WH was founded by the Austrian Research Promotion Agency (FFG) and the alpS GmbH (Project: B04 AdaptAF B)

Received: 17 January 2015; Revised: 23 October 2014; Accepted: 21 January 2015

Ecology and Evolution **2015; 5(9): 1802–1817**

doi: 10.1002/ece3.1435

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Abstract

Douglas-fir (*Pseudotsuga menziesii*) is one of numerous wide-range forest tree species represented by subspecies/varieties, which hybridize in contact zones. This study examined the genetic structure of this North American conifer and its two hybridizing varieties, coastal and Rocky Mountain, at intervarietal and intravarietal level. The genetic structure was subsequently associated with the Pleistocene refugial history, postglacial migration and intervarietal hybridization/introgression. Thirty-eight populations from the USA and Canada were genotyped for 13 nuclear SSRs and analyzed with simulations and traditional population genetic structuring methods. Eight genetic clusters were identified. The coastal clusters embodied five refugial populations originating from five distinct refugia. Four coastal refugial populations, three from California and one from western Canada, diverged during the Pleistocene (56.9–40.1 ka). The three Rocky Mountain clusters reflected distinct refugial populations of three glacial refugia. For Canada, ice covered during the Last Glacial Maximum, we present the following three findings. (1) One refugial population of each variety was revealed in the north of the distribution range. Additional research including paleodata is required to support and determine whether both northern populations originated from cryptic refugia situated south or north of the ice-covered area. (2) An interplay between *intravarietal* gene flow of different refugial populations and *intervarietal* gene flow by hybridization and introgression was identified. (3) The Canadian hybrid zone displayed predominantly introgressants of the Rocky Mountain into the coastal variety. This study provides new insights into the complex Quaternary dynamics of this conifer essential for understanding its evolution (outside and inside the native range), adaptation to future climates and for forest management.

Introduction

Within the native distribution range of a tree species, the contemporary genetic structure provides insights into a species' demographic history, colonization events as well as past or present hybridization events (Godbout et al. 2008; Cullingham et al. 2012; Semerikov et al. 2013). As a result of population history and complex interactions with the environment numerous forest tree species differentiated into subspecies, varieties and ecotypes (e.g., *Abies lasiocarpa, A. grandis, A. concolor, Larix sibirica, Pinus radiata*) with hybrids and introgressed individuals in zones of contact (Hunt 1993).

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a tree species of temperate and boreal forests with both a wide native distribution range and a large artificial range

outside its natural distribution, where it regenerates naturally (Fussi et al. 2013). It is a common northwestern American conifer covering a large latitudinal (19° to 55°) and elevation (sea level to 3260 m) range (Hermann and Lavender 1990; Kleinschmidt and Bastien 1992). Within its native range, two varieties are commonly recognized: the coastal variety (*P. menziesii* var. *menziesii*) and the interior variety (*P. menziesii* var. *glauca*) (Eckenwalder 2009). The native distribution area of var. *menziesii* covers the 2200 km coastal range from central British Columbia (BC) into central California. The var. *glauca* extends 4500 km from central BC across the Rocky Mountains into southern Mexico. Both varieties differ morphologically and ecologically. The var. *menziesii* grows faster and is considerably larger in size than the var. *glauca,* which is more shade tolerant, more cold-hardy, and more drought resistant (Eckenwalder 2009). The phylogeography within this species was refined by sequencing chloroplast DNA (cpDNA), which is paternally inherited in the Douglas-fir. The sequencing revealed three distinct phylogenetic lineages: the coastal, Rocky Mountain and Mexican lineage. Consequently, the var. *glauca* and its range were divided into the Rocky Mountain lineage (northern Rockies to southern New Mexico) and the Mexican lineage (central Mexico to Oaxaca) (Li and Adams 1989; Wei et al. 2011; Adams and Stoehr 2013).

Divergence estimates of Douglas-fir in its three main lineages (with the Rocky Mountain lineage being the oldest) differ among recent studies (Gugger et al. 2010, 2011; Wei et al. 2011). Wei et al. (2011) dated this split to have occurred from late Miocene and through the Pliocene (10–3.2 Ma), whereas Gugger et al. (2010, 2011) reported estimates from 4.372 Ma to 755 ka (from Pliocene to mid-Pleistocene). In more detail, the split of the coastal lineage from the Rocky Mountain lineage was dated back to 8.5 Ma (Wei et al. 2011) and to 2.11 Ma (Gugger et al. 2010), respectively. Through Miocene to Pliocene, western northern America experienced a series of complex geological events such as the orogeny of mountain ranges (the Sierra Nevada and Cascades), which was probably the key factor in the vicariance initiating the varietal difference between the coastal and Rocky Mountain lineage (from here on the term variety will be used for both lineages) (Gugger et al. 2010, 2011; Wei et al. 2011). Before the Last Glacial Maximum (LGM, or Late Wisconsin, 18ka), both varieties occupied distinct glacial refugia (Gugger and Sugita 2010; Gugger et al. 2010; Wei et al. 2011). Both fossil and marker based data show dissimilarities concerning the number of the last glacial refugia for both varieties. For the coastal variety, two refugia located within the current distribution area in the USA (on the Pacific coast in Oregon/Washington (OR/WA) and in California were strongly suggested by fossil records (Gugger and Sugita 2010) (Fig. 1B). Their existence was also mirrored in the phylogeographic pattern found in the terpenoids (Snajberk and Zavarin 1976). However, the two-refugium theory is in discordance with range-wide allozyme (Li and Adams 1989) or recent mitochondrial DNA (mtDNA) and cpDNA phylogeographies (Gugger et al. 2010; Wei et al. 2011), which support the one refugium hypothesis. In contrast to the coastal variety with one or two possible refugia, several refugia were proposed for the Rocky Mountain variety. The existence of three refugia situated in the Rocky Mountains of the USA was well supported by both fossil and molecular data and coalescent tests (Gugger and Sugita 2010; Gugger et al. 2010). The occurrence of other (at least 4 additional) refugia was proposed based on distribution of private cpDNA types (Gugger et al. 2010). In addition to all these refugia situated south of the Laurentide ice sheet during the LGM and within the current distribution range, Wei et al. (2011) proposed one possible glacial refugium of this variety being set outside the current distribution range, in unglaciated areas to the north of the Laurentide ice sheet.

The postglacial colonization from refugia after the LGM led to contact zones and hybridization events between the coastal and Rocky Mountain varieties along the eastern Cascades of Oregon and Washington and in the interior of BC in Canada, where a broad hybrid zone was formed (Eckert et al. 2009; Gugger et al. 2010; Wei et al. 2011).

Given the genetic complexity of the Douglas-fir (characterized by the presence of different varieties, migration from several different refugia after the LGM, intervarietal hybridization, expected interrefugial gene flow and possible existence of a northern glacial refugium outside the present distribution area), this study aimed at investigating the geographical genetic structure at the intervarietal level as well as at the level within coastal and Rocky Mountain varieties separately in order to obtain a more detailed picture on the phylogeographic pattern, selected historical demography, intervarietal hybridization and introgression patterns of Douglas-fir. Instead of using new and/or a larger number of dominant organelle loci (cpDNA and mtDNA) than used in previous range-wide phylogeographic and demographic studies (e.g., by Gugger et al. (2010) and Wei et al. (2011), we followed the recommendations of these previous studies as well as those of Eckert et al. (2009), to use biparentally inherited (codominant) nuclear DNA markers for this species. Consequently, we employed 13 highly variable nuclear microsatellite markers (nuSSRs) for genotyping 38 populations situated in the USA and Canada. The other reasons we selected these markers is that they allow Douglas-fir variety identification (Fussi et al. 2013) and the study of hybridization/introgression events in general (Lexer et al. 2010; Cullingham et al. 2012; Smith et al. 2013). Owing

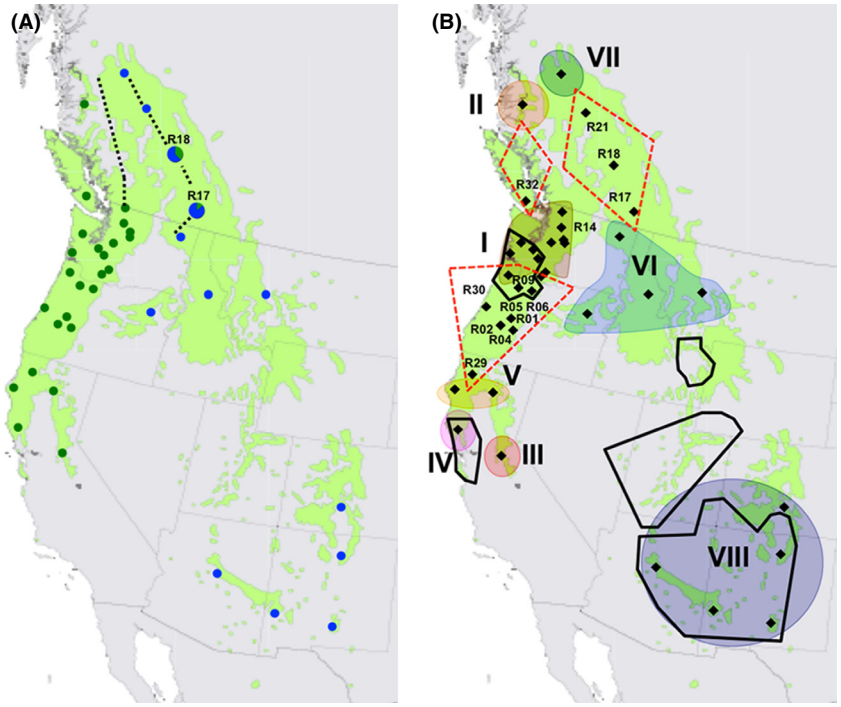


Figure 1. Distribution maps of Douglas-fir populations with their assignment to a variety (A), or to an intravarietal genetic cluster (B) as estimated by STRUCTURE. Populations R17, and R18 (map A) represent populations where individuals of both varieties, coastal (green color) and Rocky Mountain variety (blue color), were present. Dotted lines indicate the borders of the assumed intervarietal hybrid zone (von Rudloff 1973; Li and Adams 1989; Gugger et al. 2010; Wei et al. 2011). Map (B) displays eight intravarietal genetic clusters (I–VIII). Cluster-admixed populations ($0.2 < Qvar < 0.8$) are situated in overlapping (red dashed line) between two clusters I-II, I-(III+IV+V), and VI-VII. Black solid lines indicate glacial refugia based on fossil data detected by Gugger et al. (2010).

to recombination, many realizations of the demographic process can be retrieved with nuclear markers, reducing the variability of the estimates of population parameters (Lascoux et al. 2004).

We compared and evaluated the phylogeographic genetic structure revealed by SSRs with the previously identified and proposed glacial refugial history and addressed three questions on the Quaternary glacial history and colonization of Douglas-fir: (1) Did the coastal variety expand from (at least two) different glacial refugia located in the current distribution range; (2) which refugia contributed to the spread of the investigated Rocky Mountain variety after the LGM; and (3) did intervarietal F1 hybrids rather than backcrosses to one of the variety exist within analyzed populations of the hybrid zone in BC?

Materials and Methods

Sampling

A total of 766 individuals representing 38 populations from the USA and Canada and covering the natural distribution range of the coastal and Rocky Mountain variety were collected (Table S1A and Fig. S1). Higher numbers of populations were chosen to cover specific areas in OR and WA which are recommended for seed transfer to the majority of European countries (Breidenstein et al. 1990). A minimum of 20 individuals per population were used except for one population where samples from 18 individuals were collected. For 21 populations, cambium samples were obtained from Austrian provenance trials which were established by the Federal Research and Training Centre for Forests, Natural Hazards and Landscapes (Vienna, Austria) in the late 1970s using seeds from the controlled IUFRO-seed collection section (Barner 1973), or from seeds collected in natural populations by employees of the institution. For the other 17 populations, seeds were taken from collections of the USDA Forest Service-Placerville Nursery, the BC Forest Service and from R. Klumpp (University of Natural Resources and Life Sciences, Institute of Silviculture, Vienna, Austria). These were cultivated in a nursery for 2–3 months until the seedlings were approximately 5 cm in height. Both cambium samples and whole seedlings were dried in silica gel prior to the DNA extraction. The number of mother trees, the used seed collections and provenance trials originate from, are listed in the Table S1A.

DNA extraction and genotyping

The DNA was extracted from needle or cambium tissue using an OMEGA E.Z.N.A Plant DNA Kit (OMEGA Bio-Tek, Inc., Norcross, Georgia, USA) according to the manufacturer's instructions. The quality and concentration of the extracted DNA were measured on a 1.5% agarose gel electrophoresis and the Implen OD600 DiluPhotometer. For PCR reactions, the DNA was subsequently diluted with PCR water to an equal concentration of 30 $\eta g/\mu L$. A total of 17 highly polymorphic dinucleotide nuSSRs were selected from Slavov et al. (2004). These were tested on their performance (yield and specificity) on a set of 16 individuals. The finally selected 13 nuSSRs (PmOSU_2G12, PmOSU_4A7, PmOSU_3B2, PmOSU_5A8, PmOSU_2D4, PmOSU_1F9, PmOSU_3F1, PmOSU_2D6, PmOSU_1C3, PmOSU_2C2, PmOSU_3B9, PmOSU_3D5, PmOSU_2D9), which allowed clear-cut scoring and amplified one locus only, were further tested for their use in multiplex PCRs with a final arrangement into four multiplex PCR combination groups (Table S2). For simplicity, the prefix "PmOSU" is going to be omitted from now on. The selected nuSSRs reside on eight different linkage groups with the shortest distance of 15.6 (cM) between 2D9 and 3B2 from the linkage group 1 (for further details see Slavov et al. 2004). PCR amplifications were performed with the QIAGEN Type-it Microsatellite PCR Kit following the manufacturer's protocol and annealing temperature resulting from the testing (Table S2). The PCR amplifications of the multiplex PCR combination 4 turned out to be ineffective in the majority of individuals originating from five Rocky Mountain populations and consequently resulted in missing values. These PCRs were therefore repeated using a touchdown procedure with annealing temperatures of 55–45°C for 10 cycles, each 90 sec, followed by 30 cycles at 50°C, each 60 sec. NuSSRs genotypes were resolved on an ABI PRISM™ 3100 DNA Genetic Analyzer (Applied Biosystems. Inc., Foster City, California, USA). The sizing of fragments was carried out with a Genescan 3.7 and Genotyper® 2.0 software (Applied Biosystems), utilizing the internal GENESCAN™-500 ROX™ Size Standard (Applied Biosystems). Prior to performing any statistics, the genotypes were checked and corrected for null alleles and scoring errors by MICRO-CHECKER 2.2.3 using the "Brookfield 1" algorithm (Van Oosterhout et al. 2004).

Genetic diversity and differentiation

Standard descriptive genetic diversity statistics was estimated at both the locus and the population levels. Microsatellite loci of both varieties were characterized for the number of alleles (N_a), mean number of alleles (A), effective number of alleles (N_e), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), and allelic richness (A_S). They were corrected for sample size by rarefraction, with the software FSTAT v. 2.9.3.3 (Goudet 1995, 2001) and the GenAlEx v. 6.5 software by Peakall and Smouse (2006, 2012). At the population level, the descriptive statistics included calculations of allelic richness (A_S) using the approach of Szpiech et al. (2008), and estimations of expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) using the GenAlEx v. 6.5 software (Peakall and Smouse 2006, 2012). Finally, the Hardy–Weinberg (HW) exact tests were carried out for both levels (population and locus) to estimate the heterozygote deficiency with the software GENEPOP v. 4.1.4, using the default values (Raymond and Rousset 1995; Rousset 2008).

Genetic structure and clustering

The genetic structure was analyzed at two levels, at the intervarietal level, and at the intravarietal level. For the former, analyses of genetic structure were run on the entire dataset including both varieties, whereas for the latter all analyses were run on data subsets representing populations either of the coastal variety or the Rocky Mountain variety. The populations of the whole dataset were divided into two variety subsets ($K = 2$) using Bayesian admixture analysis in STRUCTURE v.2.3.4. (Pritchard et al. 2000; Falush et al. 2003, 2007). Twenty replicates of a run with a burn-in period of 50,000 and Markov chain Monte Carlo (MCMC) iterations of 100,000 were carried out based on the admixture model with correlated allele frequencies (Falush et al. 2003, 2007). These selected adjustments for the burn-in time and MCMC iterations resulted from tests optimizing the trade-off between precision and simulation time. STRUCTURE HARVESTER (Earl and von Holdt 2012) was used to generate an input file for the software CLUMPP v.1.1.2 (Jakobsson and Rosenberg 2007), which was used to estimate the mean variety-membership coefficient (*Qvar*) of each individual and population. An individual/population was declared as coastal with *Qvar* (membership coefficient) > 0.9, Rocky Mountain with *Qvar* < 0.1 and intervarietal admixed with $0.9 > Qvar > 0.1$.

The genetic population structure of both varieties was inferred using two different clustering approaches (Bayesian analysis of STRUCTURE and principal coordinate analysis [PCoa]). The Bayesian STRUCTURE analysis was run on both variety subsets separately using identical settings as described before. In addition, each subset was cleaned from possible intervarietal-admixed

individuals ($0.9 > Qvar > 0.1$) as well as from individuals of the opposite variety when present. Each K was run 20 times. As ΔK (the real number of clusters) detects the uppermost level of genetic structure where several hierarchical levels exist (Evanno et al. 2005), it was rational to carry out a hierarchical STRUCTURE analysis within both variety subsets as these represent populations originating from different refugia and might also represent populations mixed between two refugia in the zone of contact. Thus, the size of each variety subsets was reduced step by step according to the significance of the results as inferred with hierarchical analyses of the ΔK method (Evanno et al. 2005). Means of estimated intravarietal membership coefficient (*Qintra*) for each individual and population of the 20 runs per K were computed with the software CLUMPP v.1.1.2 using the Greedy option and random input orders (Jakobsson and Rosenberg 2007). The means were then plotted using DISTRUCT v.1.1 (Rosenberg 2004). The program STRUCTURE HARVESTER (Earl and von Holdt 2012) was used to estimate ΔK within the analyzed dataset. Subsequently, each population was assigned to the cluster for which its inferred individual and population ancestry (*Qintra*) was the highest. This assignment was also checked visually on DISTRUCT plots in order to evaluate the estimated *Qintra* value of populations and their composition. Although running STRUCTURE on a population level underestimates the actual number of clusters within the dataset, we preferred this strict and controllable procedure over reshuffling the individuals among clusters, which might overestimate the number of existing clusters and cause assignment problems especially for populations resulting from gene flow between refugia. If *Qintra* for one or more populations within the studied dataset was $0.2 < Qintra < 0.8$, the procedure was additionally rerun in STRUCTURE under the *locprior* using the ΔK value recommended by Evanno (Evanno et al. 2005). The remaining settings were identical to those described previously. The use of the *locprior* option is of significance when detecting genetic structures at lower levels of divergence, as expected for the coastal variety (Li and Adams 1989; Krutovsky et al. 2009) with no false positives if there is no structure present (Hubisz et al. 2009). We did not attempt to force individual populations to belong to a particular cluster as we expected that some of them might be of admixed nature because of refugia-mixed and/or variety-mixed origin. Therefore, whenever the *locprior* option led to identical results (in the assignment of populations to a cluster and/or their admixed nature) to those without this option, the assignment of populations was accepted. Populations with $0.2 < Qintra < 0.8$ were assigned to reflect cluster-mixed populations.

Subsequently, pairwise Jost's D values among all estimated STRUCTURE clusters were calculated and tested for significance using permutations in GeneAlEx 6.5 (Peakall and Smouse 2006, 2012). Jost's D (Jost 2008) is more precise in quantifying differentiation when applying highly polymorphic markers such as SSRs (Meirmans and Hedrick 2011).

As a second approach, population clustering and the relations between populations were calculated with a pairwise codominant genotypic distance followed by a covariance standardized PCoa using GeneAlEx 6.5 (Peakall and Smouse 2006, 2012). This clustering was performed for the whole dataset as well as for the two variety subsets separately using both cleaned and not cleaned datasets from intervarietal-admixed individuals and from individuals of the opposite variety. The analyses with and without the cleaned data allow estimating the influence of intervarietal-admixed individuals on the clustering. In addition to this, we tested the clustering of populations represented by intervarietal-admixed individuals only. To do so, we run PCoa with the cleaned data of the entire whole set supplemented by "new" populations. These "new" populations were built using the intervarietal-admixed individuals (with $0.4 < Qvar < 0.6$). In more detail, intervarietal-admixed individuals of R18, R21, and R39 were used to assemble such new populations a18, a21, and a39.

Intervarietal and intravarietal demographic history

Before we estimated the demographic history of genetic clusters revealed by preceding PCoa and STRUCTURE analyses, the divergence time of both coastal variety and Rocky Mountain variety was estimated. We did it by applying Approximate Bayesian Computation (ABC) analysis in DIYABC, version 2.0 (Cornuet et al. 2014) to all nuSSR loci. The intervarietal divergence was estimated using populations of the coastal (R01-R11, R16, R30, Fig. S1) and Rocky Mountain (R22-R26, Fig. S1) variety. These populations overlap with areas where Miocene and Pleistocene fossils of Douglas-fir and a refugium for each variety characterized by cpDNA were detected (Gugger et al. 2010). Thereafter, in order to clarify possible refugial origin of the discovered genetic clusters, different putative historical scenarios were tested and dated following the standard protocol of DIYABC. We were aware of the fact that a distinct genetic cluster does not immediately represent a refugial population. The analyzed populations of each cluster are shown in Table S7. In all runs, 1,000,000 datasets were simulated for each scenario. A total of 1% of the simulated datasets most similar to the observed data were used to estimate the relative posterior

probability (with 95% confidence intervals [CIs]) of each scenario via logistic regression and posterior parameter distributions according to the most likely scenario. Because DIYABC version 2.0 selects the optimized rate from a given mutation range, we assigned mutation rates of nuSSRs to vary between 10^{-4} and 10^{-2} and thus to correspond to a general mutation rate for nuSSRs (Miah et al. 2013). All intervarietal-admixed individuals were omitted from these analyses. Following parameters were estimated: divergence time (t), effective population size of each population (N_1, N_2), and effective population size of ancestor (N_a). As t was expressed in the number of generations, we used an approximate average generation time of 100 years to convert it into years, following Gugger et al. (2010, 2011).

Hybridization and introgression pattern in intervarietal-admixed populations in BC (Canada)

In order to assess whether intervarietal F1 genotypes or backcrosses exist within analyzed populations of the hybrid zone in BC (Canada), all intervarietal-admixed individuals with $0.2 < Qvar < 0.8$ as estimated in STRUCTURE analysis ($K = 2$) from studied populations in BC have been selected. Subsequently, values of the intervarietal heterozygosity (*IH*) and maximum likelihood hybrid index (*HI*) were estimated following the approach as described by Lexer et al. (2010). In more detail, we tested whether genetically admixed intervarietal individuals, previously identified by the STRUCTURE analysis, were more compatible with the simulated F1 individuals (maximum *IH* and intermediate *HI*), or represent rather recombinant hybrids and introgressants of subsequent generations (reduced *IH* and *HI* values moving toward values of the coastal or Rocky Mountain variety). In the analysis, genotypes of 500 F1 individuals were simulated with the HYBRIDLAB software v.1.1 (Nielsen et al. 2006) using 32 randomly selected individuals from a coastal variety subset with $Qvar > 0.99$, and 32 individuals of the Rocky Mountain variety subset with $Qvar < 0.01$. Then, calculations of *IH* and *HI*, including 95% CIs, were carried out and plotted for simulated and observed intervarietal-admixed individuals and parentals with R package Introgress (Gompert and Buerkle 2010) following Gompert and Buerkle (2009).

Results

Genetic diversity and differentiation

The majority of nuSSRs were variable with up to 75 alleles (N_a) observed per locus and variety (Table S3). The locus 5A8 had the lowest number of alleles represented by 13 different alleles for the coastal and nine for the Rocky Mountain variety. The observed (H_O) and expected (H_E) heterozygosities varied from 0.255 to 0.924 among 13 nuSSR loci (Table S3), and from 0.491 (R24) to 0.904 (R11) among populations for all loci (Table S1B). Both heterozygosities were highest in the population R11 (WA), which is a population of the coastal variety (Table S1B). The lowest values for H_O and H_E were found in the population of the Rocky Mountain variety R24 (New Mexico) located in the southernmost area of this variety analyzed in the present study. This population exhibited 100% missing values for three loci (2D6, 3F1, 5A). The highest values of *As* (6.5) were found in four populations of the coastal variety. The smallest values for allelic richness ($As = 5.4$ or 5.7) were calculated in populations of both varieties with the northernmost (R38, R39) or the southernmost (R34, R22-R24) distribution in the studied native range. The inbreeding coefficients (F_{IS}) were significantly positive indicating deviations from Hardy–Weinberg expectations at both population level, and also on the locus level within variety (Table S1B, Table S3). This result (1) is most likely caused by a high frequency of null alleles, allelic dropout in the nuSSR loci, and the large polymorphism and (2) is consistent with all previously published data on Douglas-fir analyzed using SSRs. In this data, individuals of a population were represented by trees or seeds collected in close or large distances (Krutovsky et al. 2009; Fussi et al. 2013). We minimized the problem with null alleles using the MICRO-CHECKER software.

Genetic structure and clustering

The genetic structure analysis performed by the software STRUCTURE allowed us to assign populations to both varieties (Fig. 1A). Except for four populations (R17, R18, R21, R39), all populations were clearly assigned either to the coastal variety with *Qvar* of > 0.9 or the Rocky Mountains variety with $Qvar < 0.1$ (Table S1B). Within the four populations, genotypes of both varieties (R17, R18) and/or larger numbers of intervarietal-admixed genotypes (R17, R18, R21, and R39) were present. Consequently, the whole dataset was divided into two variety subsets representing the coastal and the Rocky Mountain variety with 25 (R01-R16, R19, R29, R30, R32, R34-R38) and 13 populations (R17, R20-R28, R33, R39), respectively (Fig. 1A).

In the hierarchical STRUCTURE analysis, the coastal variety subset was divided into three clusters, a northern (R38), a central (R03, R07-08, R10-13, R15-16, R19) and a southern cluster (R34-37). Nine populations (R01, R02, R04-R06, R09, R14, R29, and R30) represented

cluster-mixed populations between the central and southern cluster and two (R14, R32) between central and northern cluster (Fig. 1B). The southern cluster was further subdivided into three clusters (III–V) with the populations R34, R35, and R36-37 (Fig. 1B, Table S2B). In total, five geographically and genetically distinct coastal clusters (I–V) were detected including three in California, one in BC and one between these two regions (Fig. 1B, Table S1B).

When compared to the coastal variety, the Rocky Mountain variety split into a smaller number of genetic clusters. Its 13 populations were divided into two clusters, cluster VIII (R22-R26) situated in the south of the Rocky Mountains and a cluster with the remaining populations (Fig. 1B, Table S2B). The latter was subsequently divided into clusters VI and VII. The populations R20, R27, R28, and R33 of cluster VI are distributed in the area of the Rocky Mountains in the north of the USA and the southeast of BC. The cluster VII lies to the far north of the distribution area of the species in BC and contains only the population R39. The populations R17, R18, and R21 were cluster-mixed populations between cluster VI and VII. Results of cluster heterozygosities (H_O and H_E) were similar to those calculated for populations. The highest values of H_O, H_E, and *A*s were found in the populations of cluster I, located in the northern area of Oregon and the south of Washington to the west of the Cascades. From cluster I, a decline of diversity (H_E and *A*s) was found toward the southernmost cluster III and the northernmost cluster II within the coastal variety. The same is true for the Rocky Mountain variety when comparing the values H_E and *A*s of the cluster VI with the cluster VII in the north and the cluster VIII in the south (Table S1B). As mentioned previously, we aimed for discovering robust clusters and not for the largest number of them. Nevertheless, trends of further substructuring were evident within clusters I, VI, and VIII (data not presented).

Pairwise calculated Jost's *D* differentiation values among all clusters estimated by the hierarchical STRUCTURE analysis were significantly different displaying larger values in intervarietal than in intravarietal comparisons (Table S4). For the calculations, 12 SSRs were used except the locus 5A8 which largely exhibited no amplifications (thus missing data) in populations of Rocky Mountain cluster VIII. In the intravarietal comparisons, genetic clusters isolated by largest geographic distances were at the same time the most genetically differentiated. In more detail, the largest pairwise Jost's estimates were found between the coastal variety clusters II and III with 0.580 and between the Rocky Mountain clusters VII and VIII with 0.667, respectively.

For all PCoa, we excluded the population R24 from datasets as its missing allele values in three loci could influence the calculated genetic distances. In the plots of PCoa, the presence of the individuals of the opposite variety as identified in the populations of the Rocky Mountain variety in Canada (Fig. 2) as well as the presence of intervarietal-admixed individuals within the different datasets did not influence the clustering (Fig. 3A–D, Fig. S2A–C). More specifically, the number of clusters, their distribution within the PCoa plots, and even the position of all but one (R18) populations within the plots (Fig. 3A and Fig. S2A) were similar for both cleaned and not cleaned datasets. As expected, populations represented only by intervarietal-admixed individuals (a18, a21, a39) were located in between the Rocky Mountain and the coastal variety, but closer to the Rocky Mountain variety (cluster VI and VII) of which populations they were derived from (Fig. 3B).

The PCoa at the intervarietal level in both datasets (cleaned and not cleaned) revealed more structure than the Bayesian analysis by STRUCTURE at this level. In total, three clusters were separated; two of the Rocky Mountain variety and one of the coastal variety with two populations (R35 and R38) clearly detached from the rest of this cluster (Fig. 3A). The first two PCoa axes accounted for 74.84% of genetic variance.

At the intravarietal level of the coastal variety, the Californian clusters (III, IV, and V) and the northern cluster II (R38) in BC were separated from the rest (Fig. 3C). The first two axes accounted for 52.25% of genetic variance. The PCoa of Rocky Mountain Douglas-fir clearly divided the northern cluster VII from the residual clusters VI and VIII (Fig. 3D). The first two principal components accounted for a larger fraction of the genetic variance (80.24%) within the Rocky Mountain variety when compared to populations of the coastal variety. All – but the R14 – cluster-admixed populations of the coastal and the Rocky Mountain varieties were positioned between matching clusters (Fig. S2B and C, Fig. 3C and D) and were thus in line with the results of STRUCTURE.

Intervarietal and intravarietal demographic history

Intervarietal divergence between coastal and Rocky Mountain varieties with a generation time of 100 years was estimated to have occurred 708 ka ago (95% CI: 984–316 ka). To estimate and to clarify the intravarietal demographic history of the eight genetic clusters revealed by STRUCTURE and PCoa, both varieties were treated separately. We designed putative historical scenarios for seven different groups (Fig. S3). Within each group, demographic histories for two genetic clusters were estimated. As a control, demographic history within the genetic cluster I after dividing it into two population

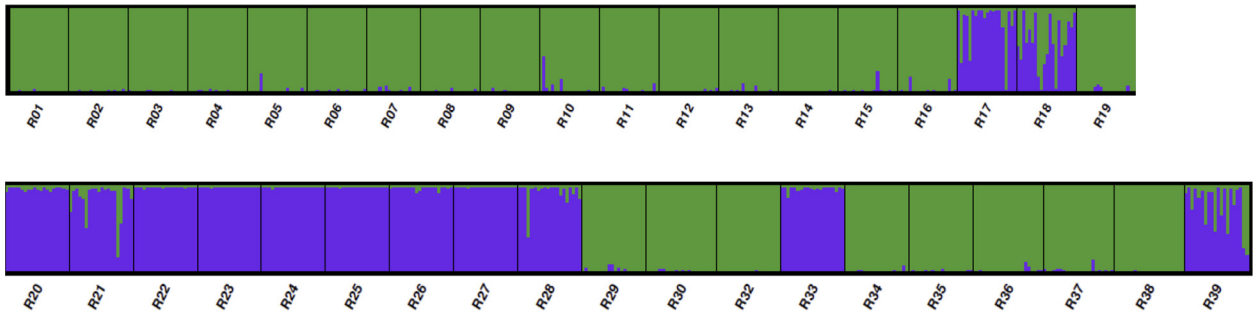


Figure 2. STRUCTURE results plotted by DISTRUCT for genetic structure at the inter varietal level (K = 2) for 766 individuals representing 28 populations (marked by R) of the coastal (green color) and Rocky Mountain variety (blue color). Individuals are grouped by populations.

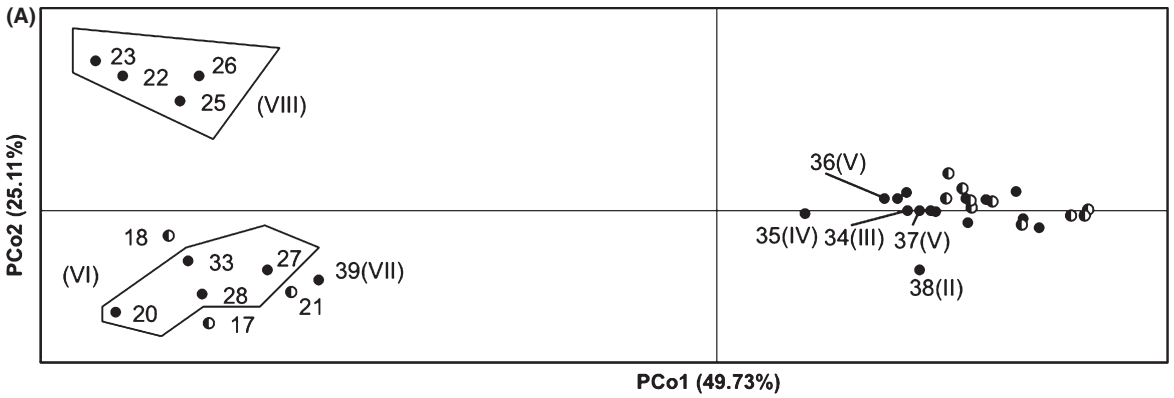
groups (I, I′) was additionally calculated. According to colonization routes after the LGM (Gugger et al. 2010), we expected the group I′ to be a post-Pleistocene (Holocene) founding derivative of the population group I. In general, three simple scenarios were simulated for all groups (Fig. 4). In scenario 1, two populations N_1 and N_2 have diverged in the past from an ancestral population N_a and in scenario 2 and scenario 3, a classic invasion history was simulated where population A was derived from population B or vice versa, respectively. More complex histories could of course be simulated, but simplicity of the model is an important criterion when selecting among competing hypotheses (Knowles and Maddison 2002). We therefore also ignored the possibility that some populations may have undergone recent expansion.

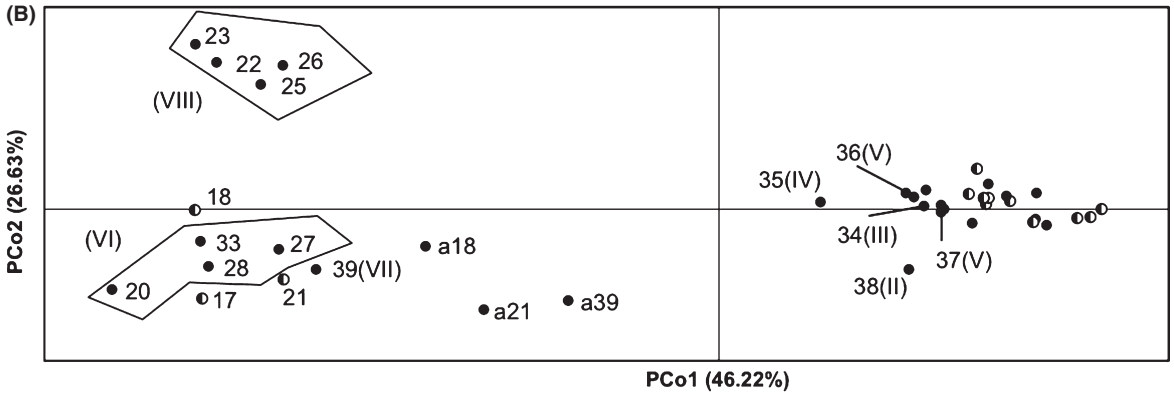
For the coastal variety, the results of ABC analysis clearly favored the hypothesis that the population group I′ and genetic clusters II, III, IV, and V were derived from the cluster I as reflected in scenario 2 (Fig. S3). The posterior probabilities for this scenario ranged between 0.38 and 0.60 (95% CI 0.64– 0.34, Table S6). Two clear trends were obvious when connecting the divergence time to a period during the Pleistocene and Holocene. The divergence of coastal populations III, IV, V, and II from the coastal cluster I distributed in western Oregon and Washington was dated back to the Pleistocene before the LGM (Table S5) with average divergence time ranging from 40.1 to 56.9 ka ago (95% CI 197.0–10.5 ka). In contrast, the group I′ is a product of the Holocene and developed from cluster I around 5.37 ka BP (95% CI 12.2–1.78 ka). For the Rocky Mountain variety, genetic cluster VIII from the southern Rockies derived from VI covering northern and Canadian Rockies around 83.9 ka BP (95% CI 230.0–22.6 ka). The cluster VII situated in BC (Canadian Rocky Mountains) was estimated to be a derivate from cluster VI with a divergence time during Pleistocene around 41.7 ka ago (95% CI 143.0–7.48 ka). The posterior probabilities for both splits were 0.70 (95% CI 0.73–0.65) and 0.44 (95% CI 0.60–0.28), respectively (Table S6).

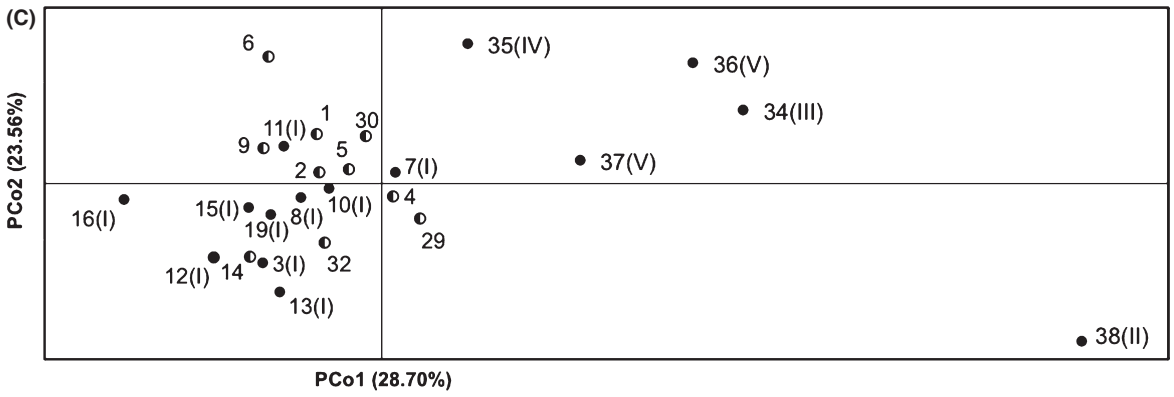
Intervarietal hybridization and intervarietal introgression

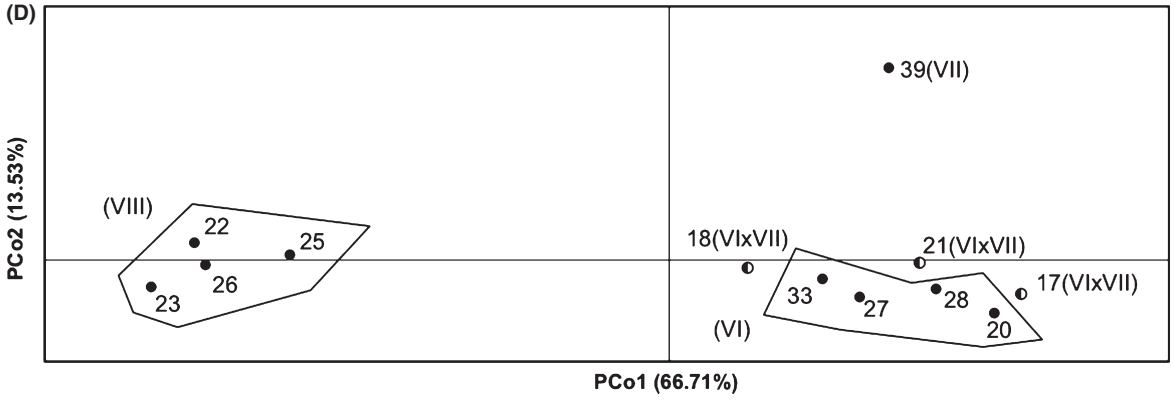
In total, 25 individuals (with $0.2 < Qvar < 0.8$) have been assigned to represent intervarietal-admixed genotypes by STRUCTURE (Fig. 2). The majority of these genotypes (21) were identified in all four populations (R17, R18, R21, and R39) located in interior BC (Fig. S1), whereas in the populations R17 and R18 individuals of both varieties were also recognized (Fig. S1, Fig. 2). The remaining four intervarietal-admixed individuals were identified in populations of coastal variety situated along slopes of the Cascades (R10, R15) in WA and in OR (R05) (Fig. S1). Within the Rocky Mountain variety, one such individual was found in the Blue Mountains of Oregon (R28). When extending the admixture boundaries ($Qvar$) for intervarietal-admixed individuals ($0.15 < Qvar < 0.85$), 12 admixed individuals could be additionally recognized within beforehand mentioned

Figure 3. Plots of principal coordinates analysis (PCoa) described by two principal coordinates (PCo1, PCo2) and explained genetic variance (%) for populations (R01-R39) without intervarietal-admixed individuals and individuals of the opposite variety for the entire dataset (A) for the entire data set and three "new" populations (a18, a21, a39) consisting of intervarietal-admixed individuals only (B), for the coastal variety (C) and the Rocky Mountain variety (D). Roman numbers (in parentheses) at the back of each population represent a membership to the particular genetic cluster as assigned by STRUCTURE analysis. Populations of identical STRUCTURE cluster are grouped together (marked areas). The cluster-admixed populations are embodied by half-filled dots. Dots with missing population numbers represent populations of the cluster I and its cluster-admixed populations.









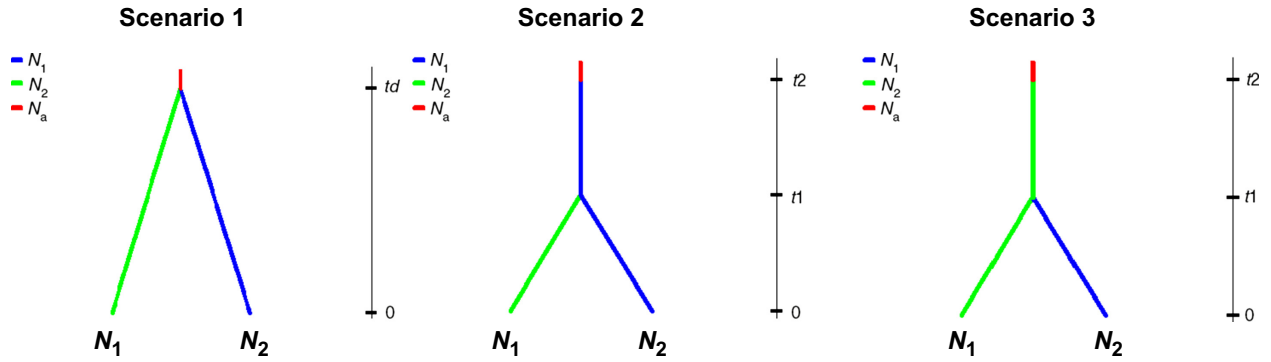


Figure 4. Three historical scenarios tested for genetic clusters of the Douglas-fir in an Approximate Bayesian Computation (ABC) as implemented in DIY ABC (Cornuet et al. 2014).

populations (R10, R17, R18, R28, and R39) with just one additional population R16 located close to R10. All are positioned in existing or possible contact regions between both varieties.

In the analysis of intervarietal heterozygosity (*IH*) versus maximum likelihood *HI*, the majority of the individuals with intervarietal-admixed genotypes from populations R17, R18, R21, R39 had a *HI* larger than 0.5 (*x*-axis in Fig. 5). The intervarietal heterozygosities (*y*-axis) were distributed below the 95% confidence intervals of simulated F1′ intervarietal hybrids (Fig. 5). The increase in the *HI* values of the majority of individuals with admixed genotypes moving toward *HI* values estimated for the coastal variety ($0.9 < HI < 1$) and their reduced *IH* pointed to their not recent origin and backcrossing to the coastal variety. Although both varieties might have come into contact at different times in Canada, there was no apparent pattern/grouping concerning *IH* and *HI* of these intervarietal recombinants and introgressants among populations (Fig. 5).

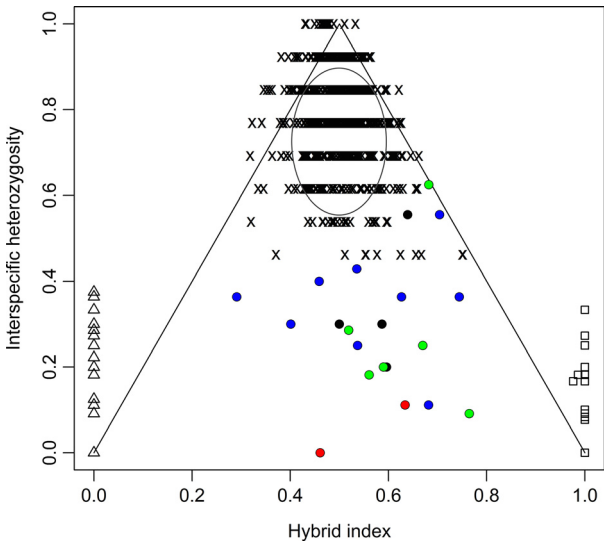


Figure 5. Intervarietal heterozygosity (*y*-axis) versus hybrid index (*x*-axis) estimated for variety-admixed genotypes from studied populations in British Columbia. Circles indicate observed values for variety-admixed individuals (red circles = R17, blue circles = R18, black circles = R21, green circles = R39); triangles indicate Rocky Mountain variety and tetragons stand for the costal variety. Crosses indicate values of simulated F1′s and the ellipse the 95% confidence intervals of simulated F1′s.

Discussion

Divergence of coastal and Rocky Mountain varieties

The intervarietal divergence time as estimated by DIYABC was placed into mid-Pleistocene (708 ka, 95% CI 984–316 ka), a more recent period when compared to the older estimates of two recent reports based on cytoplasmic (cpDNA and mtDNA) data (Gugger et al. 2010; Wei et al. 2011). In the first estimate, Wei et al. (2011) dated the divergence of the coastal variety from the Rocky Mountain variety back to the Miocene 8.5 Ma (CI 16.5–2.4 Ma). The Miocene variety divergence was also in congruence with the paleobotanical evidence of both varieties (Critchfield 1984). The second estimate based on cytoplasmic data dated the intervarietal diversification to be more recent by placing it into Early Pleistocene 2.11 Ma (CI 4.37 Ma–755 ka) (Gugger et al. 2010). Although the intervarietal nuSSR estimates of this study overlapped with those of Gugger et al. (2010) and have even showed similarity to the mid-Pleistocene intervarietal split reported in an isoenzyme study (505–315 ka) (Li and Adams 1989), our nuSSRs based estimates for the intervarietal split must be interpreted with caution. Some possible explanations for the differing estimates compared to the two cytoplasmic DNA studies are dissimilarities in the mutation rate among these marker systems and different

statistical models used. Furthermore, the rather recent nuSSR estimate of divergence was possibly affected (1) by the employment of a general and not Douglas-fir specific mutation rate for nuSSRs; (2) by the selection of only two genetic clusters for intervarietal calculations which may not cover the complex intervarietal divergence and most importantly, (3) by the fact that the algorithm used in DIYABC does not include any migration among varieties/clusters and may thus underestimate these estimates (Semerikov et al. 2013). Nevertheless, the nuSSR estimate may indicate an existing gene flow among varieties until the mid-Pleistocene. Similar to the post-Pleistocene colonization, secondary intervarietal contacts might have established during interglacials of the Pleistocene when both varieties might have periodically expanded and reduced their distribution range.

Phylogeography of the coastal variety and refugia

In contrast to all previous studies, the nuSSRs revealed more detailed genetic structure of this variety by identifying five geographically distinct clusters (Table S6, Fig. 1B). How does the distribution of these clusters and their divergence correspond to the location of fossil-determined glacial refugia and the postglacial colonization of this variety?

The allocation of the cluster I (refugial population 1) in western OR and WA was consistent with the existence of a former refugium on the Pacific coast of these states and with postglacial colonization to the south (California), to the east, where both slopes of the Cascades have primarily been colonized by it and to the north colonizing southern BC (Fig. 1B). This glacial population, which all coastal clusters diverged from, even possessed the largest diversity indices of all clusters (Table S1A) as was also suggested in the refugia concept of Hewitt (1996).

In California, where fossil records indicated the other refugium (Gugger and Sugita 2010), not one but three refugial (glacial) populations were discovered: one along the coast (cluster IV), one near the Sierra Nevada (cluster III) and one in the north (cluster V) (Fig. 1B); all with very similar Pleistocene divergence (56.9–40.1 ka, CI 197–10.1 ka) from the more northerly situated glacial population I (Table S6). Such detailed structuring has not yet been discovered in this area. However, rare alleles, different ecotypes, or a separate chemical race are acknowledged from other studies for these sites (Zavarin and Snajberk 1973, 1975; Klumpp 1999; Gugger et al. 2010). The location of the glacial population IV overlaps with Gugger et al.'s (2010) refugium located in the unglaciated San Francisco Bay area (Fig. 1B). The glacial population III and V originated from other small-scale refugia. A similar phylogeographic pattern was revealed in *Notholithocarpus densiflorus* (tanoak), a forest tree associated with the Douglas-fir in California (Nettel et al. 2009). Our hypothesis on the existence of three refugial areas in California is built on larger genetic differentiation between III and IV when compared to III and V and IV and V (Table S3), their distances in the PCo (Fig. 3C), genetic distances to cluster I, and the more ancient split of III from I (56.9 ka) than IV from I (45.9 ka) (Table S5). The range of topographic conditions in this area gives additional support for this reasoning (Fralish and Franklin 2002).

Phylogeography of the Rocky Mountain variety and refugia

When comparing the distribution of the three nuSSR-based genetic clusters (VI–VIII, Fig. 1B) to the location of the three refugia recognized by fossils (Gugger et al. 2010), then only the area of the southernmost cluster VIII with populations situated in Arizona, New Mexico and Colorado overlapped with a refugial area (Fig. 1B). Identical to the coastal variety, these southern situated glacial populations have diverged from more northerly placed populations (cluster I of the coastal v., and cluster VI of the Rocky Mountain v.) in the Pleistocene. Similarly to this result, cpDNA types of Douglas-fir populations from the southern Rockies have been estimated to be of younger origin than those found in the central US Rockies (Wei et al. 2011). The northern US Rockies cluster VI, which spread into BC and from which both the southern (VIII) and the northern glacial population (VII) have diverged, has most probably originated from one of the two central US Rockies refugia, which were described by Gugger et al. 2010 (Fig. 1B). The absence of material from these areas did not allow to clarify this.

Possible cryptic glacial refugia inside and outside the current distribution range

The existence of one distinct glacial population for each variety (cluster II and VII) situated in the north of the current distribution area of Douglas-fir in BC (Fig. 1B) led to the question from which refugia these northernmost populations in BC originated. Based on the geographic location of these populations and the divergence from the more southern refugial populations (I and VI) before the LGM (41.7 and 48.9 ka), we hypothesize that their origin is from two distinct cryptic and low-density refugia, which have escaped detection in the fossil record and thus have not been identified so far. These refugia may have been located either to the south of the Lauren-

tide ice sheet (as the suggested Rocky Mountain refugium located in the northern US Rockies (Gugger et al. 2010)), but at higher latitudes than refugia for glacial populations I and VI or even in unglaciated areas located to the north of the ice sheet, in climatically favored microsites. Without backing from fossil evidence and an extensive sampling in Canada and the northern US Rockies, however, these populations cannot be attributed to either of them with confidence.

The existence of northern refugia situated to the north of the ice sheet, such in the Queen Charlotte Islands and in Beringia, has been inferred by paleorecords and/or suggested by genetic surveys for other temperate and boreal conifers co-occurring with Douglas-fir such as *Tsuga mertensiana* (Hansen and Engstrom 1996), *Pinus contorta* (Hansen and Engstrom 1996; Godbout et al. 2008; Strong 2010), *P. monticola* (Kim et al. 2011), *Picea sitchensis* (Gapare et al. 2005), *P. glauca* (Anderson et al. 2006, 2011), and *P. mariana* (Gérardi et al. 2010). Even for Douglas-fir and for the Rocky Mountain variety in particular, Wei et al. (2011) hypothesized on such possible northern refugium after the detection of an endemic cpDNA-type with unique and broad presence in BC. For the coastal variety, an own Douglas-fir ecotype classified by isoenzymes and geographic structure was found in the geographic area (the Chantslar lake, BC) of the northern cluster II (R38) (Klumpp 1999). If such northern refugia existed, then postglacial migration rates were even lower than their recent estimates (50–165 m/year, Gugger et al. 2010) and far below the migration rates required to keep up with future climate projections (1000 m/year, McLachlan et al. 2007).

Intervarietal contact/hybrid zones and introgression

Canada was colonized by four genetically distinct populations, two of each variety (I, II, VI, VII) which expanded from four separate refugia. The postglacial colonization which brought both varieties to a contact at the present eastern border of the hybrid zone (reflected by the coexistence of both varieties within populations R17, R18) followed by intervarietal hybridization and predominant unidirectional introgression into the coastal variety rather than assortative mating of F1 hybrids (R17, R18, R21, and R39) led to a 450-km wide hybrid zone in BC (Gugger et al. 2010) (Fig. 1A). A similar pattern was revealed using dominant markers by identifying an extensive and preferential pollen flow from east to the west of this hybrid zone, while seed-mediated gene flow remained geographically restricted (Gugger et al. 2010). In tree species, a high prevalence of introgressed genotypes over "true" hybrids is frequent and was also reported for other "Canadian" conifer hybrid zones such as for *Picea mariana* and *P. rubens* in northeastern Canada (Perron and Bousquet 1997), *Pinus contorta* and *P. banksiana* in Alberta in western Canada (Cullingham et al. 2012; Godbout et al. 2012), or *Picea sitchensis* and *P. glauca* in northern BC (Hamilton and Aitken 2013). Nevertheless, the structure of the Douglas fir hybrid zone in Canada is more complex than previously described. From the four populations (two of each variety) which colonized BC in the Holocene, three of them contributed to the intervarietal gene flow in addition to the intravarietal (intercluster) gene flow (cluster VI and VII with R18 and R21, Fig. 1B). Further studies with more detailed sampling design is needed to investigate this complex situation in BC.

In addition to the hybrid zone described for Canada, the postglacial migration of both varieties led to two additional contact/transition zones in central Oregon and in the eastern Cascades of northern Washington. In central Oregon, when merging all published data (Li and Adams 1988, Gugger et al. 2010; Wei et al. 2011) including nuSSRs of this study, the transition zone covers an area from the eastern slopes of the Middle Cascades to the central part of the Blue Mountains in Oregon. In the second transition zone in the Cascades of WA, the Rocky Mountain variety spread into the coastal by pollen (Wei et al. 2011). An equivalent pattern of gene flow has been identified by SNPs (single nucleotide polymorphisms) associated with the cold-hardiness of the Douglas-fir growing east of the Cascade crest in Washington. Despite the possible recent intervarietal contact in the Cascades, cold-adapted alleles which originated and introgressed from the Rocky Mountain variety have been discovered in individuals of the coastal variety (Eckert et al. 2009).

Conclusion and Outlook

The codominant SSRs markers allowed novel insights into the Quaternary glacial epoch dynamics of this tree species including (1) the estimation of glacial history and divergence times for six distinct genetic clusters (populations), (2) the identification of so far the most comprehensive phylogeographic structure of the coastal variety, and finally, (3) the revealing of a complex postglacial recolonization in BC.

Existing phylogeographic patterns allow light to be shed on the impact of past climatic cycles on species distributions, which in turn may help to facilitate predictions on how species will respond to future climates. Recent distribution models which incorporated intraspecific variation (taken to be presence of different subspecies within a species, or provenance trial data) resulted in a different (and

more optimistic) projection of future suitable habitats than the models that treated species as single entities (e.g., Oney et al. 2013). Such projections may differ even more when incorporating more details such as glacial and postglacial populations and the hybridization events as revealed in this study.

And finally, the Douglas-fir is only one of several broadly distributed and economically important forest tree species (such as *Abies grandis, Picea sitchensis*, *Pinus contorta*, *Robinia pseudoacacia*, *Quercus rubra)* with extensive introduction outside their native ranges, where they sometimes naturalize or even become invasive (Call and Nilsen 2005; Kota et al. 2007; Traveset et al. 2008; Cierjacks et al. 2013; Radtke et al. 2013). Douglas-fir was introduced in numerous countries worldwide (e.g., New Zealand, Chile, Argentina, Australia, France) (Bastien et al. 2013). In European forestry, this conifer is seen as an alternative to some native tree species mainly because of its stable growth potential even under the dryer conditions, which are predicted for the future (IPCC 2007; Eilmann et al. 2013). Detailed knowledge of the genetic structure and hybridization/introgression patterns of this tree in northern America is crucial (1) for understanding how this species may evolve in artificially variety-mixed stands existing in Europe (Fussi et al. 2013) and (2) for planning for future climates and stable production while considering that imported seeds from areas in interior BC and along the eastern Cascades in OR and WA are likely to be of intervarietal-admixed character and therefore not "pure" varieties.

Acknowledgments

The authors are grateful to Rupert Seidl, Adam Moreno and three anonymous reviewers for providing constructive comments that improved this manuscript. For financial support, we want to thank to the following enterprises, state and private landowners: the Austrian Science Fund (FWF) (Project-ID: P26504), the Austrian Research Promotion Agency (FFG), the alps GmbH (Project: B04 AdaptAF B), the Austrian Chamber of Agriculture, the Chamber of Agriculture of Lower Austria, Austrian Federal Forests, Land and Forstbetriebe Niederösterreich, the Metternich'sche Forstamt Grafenegg, the Forstamt Stift Göttweig, the Esterhazy Betriebe GmbH, the LIECO GmbH, the Montecuccolli Gut Mitterau, the Fürst Starhemberg'sche Familienstiftung, the Gutsverwaltung Bubna-Litic, the Hatschek Forste, the Hoyos'sche Forstverwaltung Horn, the Forstamt Ottenstein for financial support. Also, we would like to thank Lambert Weissenbacher from the Federal Research and Training Centre for Forests, Natural Hazards and Landscape for his help with the sample collection.

Conflict of Interest

None declared.

Data Archiving Statement

Sampling locations: supplementary material.

Microsatellite genotypic data will be submitted in STRUCTURE format to Dryad.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Distribution map of Douglas-fir populations (R01-39) within its natural range in Northwest America.
Figure S2. Plots of Principal Coordinates analysis (Pcoa) described by two principal coordinates (PCo1, PCo2) and explained genetic variance (%) for populations (R01-R39) with inter-varietal-admixed individuals and individuals of the opposite variety (A) for the entire data set, (B) for the coastal variety (C) and the Rocky Mountain variety.
Figure S3. Different scenarios arranged into 7 groups to analyse the demographic history of the coastal and Rocky mountain variety.
Table S1A. Population number (Pop.-Nr.), geographic origin (state-prov., and coordinates inclusive altitude), number of collected individuals (N), number of mother trees (NM): mostly 15 (Kleinschmidt and Bastien 1992) (A), >20 (B), >50 (C), ~ 50 (D), unknown (E), 15 from 5 stands (F), 15 (G).
Table S1B. Genetic cluster (C) membership (I–IX based on STRUCTURE), population number (Pop. -Nr.), variety assignment (*Qvar*), intra-varietal assignment (*Qintra*) to the genetic cluster (C) marked in bold, heterozygosities *H*o and *H*E, allelic richness (*A*s), inbreeding coefficent (*F*IS).
Table S2. nuSSRs loci, their multiplex combination (1–4), and used (optimized) annealing temperature.
Table S3. Genetic variability at 13 nuclear microsatellite in coastal and Rocky-Mountain Douglas-fir, including number of alleles (*Na*) mean number of alleles (*A*), effective number of alleles (*N*e), observed heterozygosity (*H*O), expected heterozygosity (*H*E), inbreeding coefficient (*F*IS), allelic richness (*A*s).
Table S4. Pairwise Jost's D values among all nine genetic Douglas-fir clusters based on 12SSRs (without locus 5A8) below diagonal.
Table S5. Estimations of the posterior distributions of parameters revealed from the Approximate Bayesian Computation for the best scenario.
Table S6. Posterior probabilities for the scenarios with the highest posterior probability of 10,000 set of summary statistics most similar to the observed data through logistic regression.
Table S7. Populations of each cluster/group for DIYABC.

9.2 Publikation II

Hintsteiner WJ, Van Loo M, Neophytou C, Schueler S, Hasenauer H (2018) The geographic origin of old Douglas-fir stands growing in Central Europe. European Journal of Forest Research 137(4):447-461

The geographic origin of old Douglas-fir stands growing in Central Europe

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European Journal of Forest Research

ISSN 1612-4669
Volume 137
Number 4

Eur J Forest Res (2018) 137:447-461
DOI 10.1007/s10342-018-1115-2

Springer

European Journal of Forest Research (2018) 137:447–461
https://doi.org/10.1007/s10342-018-1115-2

ORIGINAL PAPER

The geographic origin of old Douglas-fir stands growing in Central Europe

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Received: 18 November 2017 / Revised: 12 April 2018 / Accepted: 17 April 2018 / Published online: 3 May 2018

Abstract

Since the nineteenth century, Douglas-fir seed sources have been widely used for establishment of forest stands outside its natural distribution range. In Europe, some of these old Douglas-fir stands are registered as seed stands and provide seed sources for nurseries, although it is unclear from which region in North America they originate. In recent years, the interest in planting Douglas-fir has increased substantially because the species is seen as a potential adaptation option to climate change. This makes the assignment of European Douglas-fir stands of unknown seed origin to their geographic origin in North America increasingly important, because the genetic quality of these plantations must be guaranteed. In this study, we use 13 nuSSR loci to investigate the origin of 67 Austrian and German Douglas-fir stands of unknown origin. We performed a hierarchical Bayesian cluster analysis using 38 native Douglas-fir populations. The resulting clusters are used as reference populations to assign the 67 Central European Douglas-fir stands from Austria and Germany planted more than 80 years ago. Our results suggest that the majority of our investigated Douglas-fir stands come from central Washington (USA), the recommended seed zones for Central Europe. Some stands were located outside the suggested area, e.g. central Oregon and Santa Fe (New Mexico). The accuracy assessment of our approach revealed the best performance for the highest hierarchical level, e.g. assigning populations either to the coastal or the Rocky Mountain variety. As expected, the uncertainty increases with decreasing hierarchical level. The final assessment, if an admixture of seed sources within the European Douglas-fir stands is evident suggests that 23 of the Douglas-fir stands show an admixture which was not detected in our Douglas-fir reference populations growing in the natural distribution range.

Keywords *Pseudotsuga menziesii* · Genetic assignment · Nuclear microsatellites · Inter-varietal structure

Introduction

Europe has introduced Douglas-fir 190 years ago. In the first 140 years, the importance of the geographic origin of seeds or provenance was neglected and thus, Douglas-fir plantations established prior to the 1980s are mostly of unknown origin (Bastien et al. 2013). However, a number of these "unknown" populations were selected and registered as seed stands mainly because of their superior growth performance. Today, these "unknown" stands as well as "established" plantations are the main European Douglas-fir seed sources (e.g. Kohl and Nather 1991; Konnert 2009; Rau 2009).

The use of the best provenance is important for achieving high productivity rates and to ensure a high adaptive capacity. Provenance trials have shown that not only height and diameter growth increment, but also adaptive relevant characters are under strong genetic control. Adaptive characters

Communicated by Christian Ammer.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10342-018-1115-2) contains supplementary material, which is available to authorized users.

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include, for example, water-use efficiency effecting the drought tolerance (Darychuk et al. 2012) and bud phenology, which is related to frost hardiness (Eckert et al. 2009). In addition, a shift in the suitable plantation regions within Europe by provenance is expected due to climate change (Chakraborty et al. 2016). Consequently, the origin of European Douglas-fir stands, and particularly those that serve as seed stands, are of high interest.

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] is a North-Western American coniferous tree species covering a large latitudinal (19° to 55°) and elevational range (from sea level to 3260 m) (Hermann and Lavender 1990; Kleinschmit and Bastien 1992). Douglas-fir was introduced to many countries worldwide (e.g. New Zealand, Chile, Argentina, Australia, France), mainly because of its growth potential, robustness and timber quality (Holubčík 1968). In Europe, Douglas-fir covers about 750,000 ha, which is the second largest forest area of a non-native conifer tree species (Köble and Seufert 2001) and the largest Douglas-fir area outside its native range (Bastien et al. 2013). In recent years, Douglas-fir has become increasingly interesting because of its growth rates and its resilience versus drought stress. Douglas-fir is considered an additional potential species for adapting European forests to expected changes in climate (IPCC 2007; Eilmann et al. 2013).

The two varieties of Douglas-fir, the coastal (*P. menziesii* var. *menziesii*) and the Rocky Mountain (*P. menziesii* var. *glauca*) variety, can be distinguished based on their morphological (Allen 1960; Dunlap 1964), population genetic (Li and Adams 1989; Klumpp 1999; van Loo et al. 2015; Neophytou et al. 2016), physiological (Pharis and Ferrell 1966; Lavender and Overton 1972) and growth differences (Kleinschmit and Bastien 1992; Chakraborty et al. 2015). Genetic and paleobotanical evidence supports that the two varieties diverged due to geographic isolation during Pleistocene glacial periods (Gugger and Sugita 2010; Gugger et al. 2010; van Loo et al. 2015). The post-glacial colonisation from their refuges resulted in inter-varietal contact and admixture zones located east of the Cascades of Oregon and Washington (Eckert et al. 2009; Gugger et al. 2010; Wei et al. 2011; van Loo et al. 2015) including a 450 km hybrid zone in British Columbia (Gugger et al. 2010; van Loo et al. 2015).

Within its natural distribution area, Douglas-fir grows on aFalush wide range of site conditions. This has led to provenance trials in several countries across Europe (Barner 1973; Rehfeldt 1979, 1983; Stephan 1980; Campbell 1991; Kleinschmit and Bastien 1992; Konnert and Ruetz 2006; Weißenbacher 2008; Eilmann et al. 2013), aiming to explore the growth properties of different provenances under various climatic conditions. These studies showed differences among the two varieties and within the intravarietal level. In Central Europe, the coastal variety and provenances from Oregon to the west of the Cascade Range show the best growth performance. The Rocky Mountain variety is only recommended for some European countries with continental climate influence, e.g. in countries like Sweden, Finland or Slovakia (Holubčík 1974, 1980; Martinsson 1990; Tigerstedt 1990).

With representative population data from the original distribution range of a species (Cornuet et al. 1999), it is possible to assign populations or individuals of unknown origin to their region of origin (Cornuet et al. 1999) using highly variable molecular markers such as microsatellites (Simple Sequence Repeats; SSRs). Such assignment methods have often been used for tracing back illegal timber trade activities (Lowe and Cross 2011; Jolivet and Degen 2012; Degen et al. 2013; Nazareno and Dos Reis 2014). The accuracy of such methods depends on the genetic differentiation among the reference populations (Cornuet et al. 1999). Thus, it is important that any investigation starts with a genetic clustering and differentiation for the reference population prior to the assignment test. Pooling populations to one cluster based on unsupervised clustering methods may improve the accuracy of the method (Baudouin et al. 2004; García et al. 2006; Nazareno and Dos Reis 2014).

Population genetic analyses of European Douglas-fir stands have differentiated between the two varieties (Konnert and Ruetz 2006; Fussi et al. 2013) but have never used reference populations from its native range (Konnert and Ruetz 2006; Fussi et al. 2013). Given the large ecological and growth differences of provenances within a variety, a refinement in the assignment to intravarietal origins is of interest.

The purpose of this study is to take advantage of a large set of genotyped reference Douglas-fir populations from the native range and develop a new assignment method based on a marker set of 13 nuSSRs to trace back the native geographic origin of 67 Austrian and German Douglas-fir stands of unknown origin, mainly planted more than 100 years ago. Our reference dataset consists of 38 Douglas-fir populations covering the entire distribution range of the coastal and Rocky Mountain varieties across USA and Canada. We are specifically interested in

(i) the pattern of genetic differentiation (clustering) among reference populations within the native range of Douglas-fir,
(ii) the accuracy of the assignment in relation to the inferred clusters at different hierarchical levels, and
(iii) the assignment of 67 Austrian and German Douglas-fir seed stands of unknown origin including the identification of potentially admixed (i.e. seed source mixed) populations.

Materials and methods

For our analysis, two different datasets are used: (i) reference data covering the full range of the natural distribution of Douglas-fir, and (ii) Douglas-fir stands from Austria and Germany of unknown origin. Note for each of these data, the same DNA data extraction method and genotyping was applied.

Study populations

Reference data from USA and Canada

We analyzed 766 individuals from 38 populations (R01-R30, R32-R39, note R31 is missing), covering the natural distribution range of Douglas-fir within the US and Canada (Table 1; Fig. 1; van Loo et al. 2015). A higher sampling density was chosen for Oregon (OR) and Washington (WA) because these areas were traditionally the main seed collection zones for Douglas-fir plantations in Europe (Breidenstein et al. 1990). For each reference population (except population R07 with 18 individuals),

Table 1 Population name (Pop.), number of individuals (*N*), coordinates and country of each reference population and the European populations

Pop.	*N*	Latitude	Longitude	Country	Pop.	*N*	Latitude	Longitude	Country	Pop.	*N*	Latitude	Longitude	Country
R01	20	44,41	– 122,47	US-OR	R37	22	40,14	– 124,05	US-CA	FF02	16	48,33	8,35	DE
R02	20	44,22	– 122,07	US-OR	R38	22	52,35	– 126,03	CA-BC	FF03	16	48,33	8,35	DE
R03	20	45,77	– 123,22	US-OR	R39	20	54,04	– 125,34	CA-BC	FWW01	26	47,82	9,72	DE
R04	20	44,50	– 122,00	US-OR	S01	20	48,45	16,45	AT	FWW02	26	47,77	9,72	DE
R05	20	45,40	– 121,38	US-OR	S02	20	47,60	16,34	AT	GW01	18	48,35	15,60	AT
R06	20	45,38	– 122,30	US-OR	S03	20	47,56	16,32	AT	OSF01	26	49,01	10,46	DE
R07	18	46,37	– 123,73	US-WA	S04	20	48,51	15,72	AT	OSF02	26	48,99	10,55	DE
R08	20	47,25	– 123,42	US-WA	S05	20	48,51	15,73	AT	OSF03	26	49,03	10,58	DE
R09	20	45,97	– 121,53	US-WA	S06	20	48,51	15,72	AT	TT01	26	48,00	9,47	DE
R10	20	46,04	– 121,44	US-WA	S07	20	48,16	14,98	AT	TT02	26	48,03	9,42	DE
R11	20	46,75	– 122,13	US-WA	S08	20	48,18	15,03	AT	TT03	20	48,07	9,50	DE
R12	20	48,30	– 121,60	US-WA	S09	20	47,90	14,82	AT	TT04	19	48,09	9,58	DE
R13	20	48,26	– 121,56	US-WA	S10	20	47,02	15,59	AT	TT05	26	48,14	9,57	DE
R14	20	48,65	– 121,72	US-WA	S11	20	48,75	15,00	AT	TT06	26	48,22	9,75	DE
R15	20	47,54	– 121,55	US-WA	S12	20	48,34	14,72	AT	UF01	24	48,05	9,26	DE
R16	20	46,50	– 121,89	US-WA	S13	20	48,32	14,77	AT	UF02	10	48,22	9,18	DE
R17	20	49,50	– 117,27	CA-BC	S14	20	48,17	13,60	AT	UF03	26	48,75	8,32	DE
R18	20	51,17	– 119,54	CA-BC	S15	20	47,93	13,41	AT	UF04	26	48,04	7,76	DE
R19	20	49,00	– 121,75	CA-BC	S16	20	48,33	14,78	AT	UFX1	24	48,40	8,64	DE
R20	20	48,60	– 118,73	US-WA	S17	20	48,52	15,76	AT	UFX2	25	48,40	8,64	DE
R21	20	52,69	– 122,43	CA-BC	S18	20	48,51	15,72	AT	UFX3	26	48,21	9,22	DE
R22	20	34,93	– 111,35	US-AZ	S19	20	47,64	16,44	AT	UFX4	25	48,16	9,10	DE
R23	20	33,43	– 108,60	US-NM	AS01	21	50,48	6,37	DE	UFX5	26	48,22	9,09	DE
R24	20	32,83	– 105,55	US-NM	AS02	26	50,52	6,46	DE	WBR01	25	51,08	8,27	DE
R25	20	35,75	– 105,83	US-NM	AZ02	19	49,04	12,56	DE	WBR02	25	51,08	8,26	DE
R26	20	37,95	– 105,07	US-CO	AZ04	20	48,64	13,01	DE	WBR03	26	51,09	8,46	DE
R27	20	46,47	– 115,35	US-ID	AZ06	18	48,41	13,46	DE	WBR04	26	51,11	8,49	DE
R28	20	44,95	– 118,15	US-OR	AZ07	19	48,24	13,45	DE	Z01	20	48,28	12,67	DE
R29	20	40,85	– 123,43	US-CA	BL01	22	48,33	15,77	AT	Z02	20	48,28	12,67	DE
R30	22	44,38	– 123,88	US-OR	BL02	22	48,32	15,77	AT	Z03	20	48,32	12,68	DE
R32	20	49,10	– 124,03	CA-BC	CF01	20	51,03	8,48	DE	FB01	26	47,60	16,34	AT
R33	20	46,99	– 110,70	US-MT	CF02	15	50,94	8,39	DE	BH	23	48,70	15,58	AT
R34	20	37,92	– 120,05	US-CA	CF03	26	50,45	9,39	DE	FB	20	48,70	15,61	AT
R35	20	39,87	– 122,67	US-CA	CF04	26	50,65	9,47	DE	HW	21	48,62	15,53	AT
R36	22	40,36	– 121,83	US-CA	FF01	26	47,88	8,81	DE	SR	22	48,78	15,57	AT

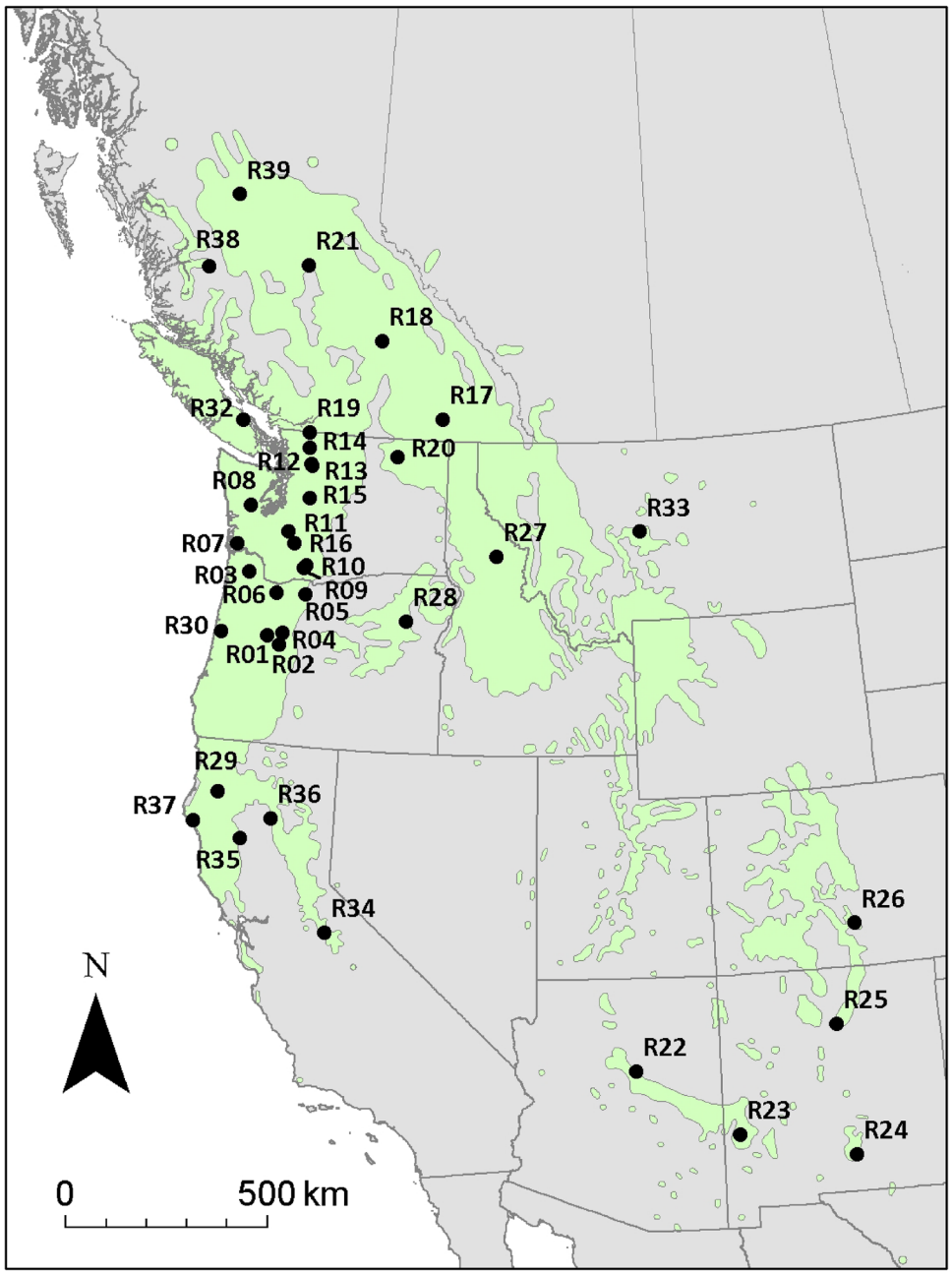

Fig. 1 Distribution map of Douglas-fir populations (R01–R39) within its natural range in Northwest America

20–22 individual trees were randomly sampled. For 21 of our 38 populations, cambium samples from provenance trials were provided by the Austrian Research Centre for Forests (BFW), Vienna, Austria. These trials used either seeds from the IUFRO-seed collection section (Barner 1973), or seeds collected in natural populations in the US (see Chakraborty et al. 2015). The seed for the remaining 17 populations, were obtained directly from the USDA Forest Service-Placerville Nursery in the US, and the British Columbia Forest Service, Canada. These seeds were cultivated for 2–3 months in the nursery until the seedlings reached approximately 5 cm in height.

Populations of unknown origin

Populations of unknown origin comprise 67 adult Douglas-fir stands in Austria and Germany (Table 1). In the following, we will refer to these stands as European stands. The oldest of these stands were established in the nineteenth century. Again, cambium samples of 10–26 adult trees per stand were randomly collected, so in sum we obtained samples of 1469 different individuals. Whenever possible, trees with high phenotypic variation were selected to ensure that both Douglas-fir varieties are captured in case adult stands originate from mixed seed collections.

DNA extraction and genotyping

Collected material (cambium samples and cultivated seedlings) of our 38 Douglas-fir reference populations (R1–R30 and R32–R39) and the 67 European Douglas-fir stands was dried and stored in silica gel prior to DNA extraction. The DNA was extracted from leaf or cambium tissue using an OMEGA E.Z.N.A Plant DNA Kit according to the manufacturer's instructions. For the population assignment, 17 dinucleotide nuSSRs from Slavov et al. (2004) were tested on a set of 16 trees to establish a reliable set of nuclear SSRs with sufficiently high polymorphism.

These tests resulted in 13 highly polymorphic and unlinked nuSSRs (Slavov et al. 2004) which were arranged into four multiplex PCR combinations (Online Resource 1). For further details on SSR selection, we refer to van Loo et al. (2015). Polymerase chain reaction (PCR) amplifications were performed with the QIAGEN Type-it Microsatellite PCR Kit following the manufacturer's protocol and annealing temperature resulting from the testing (Online Resource 1). PCRs resulting in missing values were repeated using a touch-down procedure with annealing temperatures of 55–45 °C for 10 cycles, each 90 s, followed by 30 cycles at 50 °C, each 60 s. NuSSRs genotypes were resolved on an ABI PRISM™ 3100 DNA Genetic Analyzer (PE Applied Biosystems. Inc.). The sizing of fragments was carried out with a Genescan 3.7 and Genotyper® 2.0 software (PE Applied Biosystems.Inc.), utilising the internal GENESCAN™-500 ROX™ Size Standard (PE Applied Biosystems. Inc.). Allele binning was carried out automatically using the software TANDEM (Matschiner and Salzburger 2009) for both American reference and European populations.

The reference populations are identical to those used in the study by van Loo et al. (2015), where the allele binning was performed manually. Here, we repeat allele binning using an automated method (TANDEM) to optimize the consistency of scoring. The resulting genotypes after allele binning were used for data analysis.

Data analysis and results

Population structure within the reference populations

We started our analysis by performing a Bayesian cluster analysis of the 38 reference populations from North America using the software STRUCTURE v.2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007) to infer genetic differentiation and genetic relationships among them. The applied software STRUCTURE uses Markov Chain Monte Carlo procedures (MCMC) for assigning individuals to each one of K predefined clusters based on the individuals' multilocus genotype. The program assigns each individual a membership proportion (Q value) to each one of the clusters. For all runs, we chose the admixture model and correlated allele frequencies. We performed 20 independent runs for each K between 1 and 10. For each run, we applied 50,000 burn-in replications followed by 100,000 MCMC iterations. We repeated the procedure using the *locprior* option (Hubisz et al. 2009) because this option is considered to be sensitive in detecting minor differences in the genetic structures which we expected for populations within the coastal variety (Li and Adams 1989; Hubisz et al. 2009; Krutovsky et al. 2009). The STRUCTURE analysis runs were performed with the program STRAUTO v1.0, which allows automation and parallelization of multiple STRUCTURE runs (Chhatre and Emerson 2017).

We defined the optimal number of clusters K, based on two criteria: (i) Maximisation of the statistic ΔK (Evanno et al. 2005), which is based on the second order rate of change of log likelihood of the data for consecutive K values. According to the Evanno et al. (2005), the K value corresponding to the maximum ΔK denotes the uppermost hierarchical level. For ΔK calculation we used the program STRUCTURE HARVESTER (Earl and von Holdt 2012). (ii) Unimodality among runs for a particular K, i.e. each one of the 20 runs for a particular K should lead to the same clustering solution. We controlled this by processing the multiple runs using the online platform CLUMPAK (Kopelman et al. 2015).

Performing a single cluster analysis for the whole sample may underestimate the "true" number of clusters, because smaller clusters may remain undetected, especially if sampling intensity varies among clusters and / or if hierarchical phylogenetic structure occurs within clusters (Neophytou 2014; Janes et al. 2017; Wang 2017). We performed the hierarchical analysis by repeating the Bayesian clustering within the inferred clusters, as recommended by Pritchard et al. (2010) and Vähä et al. (2007). Finally, we addressed label switching among clusters and average Q values among runs, by applying CLUMPAK after each analysis. CLUMPAK implements the default algorithms of the CLUMPP method (Jakobsson and Rosenberg 2007).

We performed the hierarchical analysis according to the following steps: (i) chose the optimal K value as described above, (ii) assign populations to each one of the K-clusters, using a threshold value of $Q > 0.60$, (iii) check whether the clustering pattern with the use of *locprior* was consistent with the analysis without locprior; (iv) If the analysis with *locprior* option led to the same clustering pattern as without any *prior*, we reiterated the procedure within each of the resulting K-clusters.

We stopped the procedure (a) if each single population was assigned to one single cluster (i.e. no longer possible to infer further population genetic structure), (b) if populations

were not split into at least two clusters, (c) if there was inconsistency of the clustering pattern with *locprior* versus without prior (which indicates that population genetic structure is very weak).

The implementation of the hierarchical cluster analysis as described is shown in Fig. 2, which was created with the software DISTRUCT (Rosenberg 2004). The first analysis including all samples resulted in the separation of the two varieties. We defined this hierarchical level of clustering as 'hierarchical level 1' (HL1). Subsequently, the varieties were subdivided into two further clusters, each at the 'hierarchical level 2' (HL2; clusters 1–4). After finishing the iterative procedure, we were left with twelve clusters (I–XII) across both varieties at the 'hierarchical level 3' (HL3). The spatial distribution of clusters in the native range for each hierarchical level is presented in Fig. 3.

Accuracy assessment through self-assignment tests within the inferred clusters

An important step of our analysis is to assess the accuracy of the assignment to clusters/populations at each hierarchical level. Clusters at a higher hierarchical level display a higher genetic differentiation which suggests a higher accuracy in the assignment. For example, a European Douglas-fir stand can be assigned more accurately to one of the two varieties (coastal or Rocky Mountain) as well as to one of the four major clusters (HL2), than to a single reference population within a variety.

We implemented the self-assignment tests for genotypes of known origin (North American) prior to the assignment of the unknown individuals/populations to assess the assignment accuracy for each of the aforementioned hierarchical levels. The software GENECLASS2 (Piry et al. 2004) was applied and each individual tree was assigned to one of the reference populations. Individuals to be assigned are excluded from the reference dataset (leave-one-out-approach) during the computation. This resulted in a percentage of correctly assigned individuals (*CA*) and a Quality Index (*QI*) defined as the mean value of the assignment scores of each individual within the population from which it was sampled (Piry et al. 2004).

We ran the self-assignment tests by computing the probability of an individual tree to belong to a population within the hierarchical levels using the computation criteria according to Rannala and Mountain (1997). The simulation algorithm of Paetkau et al. (2004) with a resampling of 10,000 simulated individuals and a Type I error (alpha) of 0.01 (Cornuet et al. 1999; Paetkau et al. 2004) was used.

We repeated the analyses at all hierarchical levels. At HL1, we defined two reference populations corresponding to each one of the two varieties based on the results of the STRUCTURE analysis (see Fig. 2). At HL2, we performed the tests within each variety. For the Rocky Mountain variety, we defined clusters 3 and 4 as reference populations. For the coastal varieties, we defined 3 reference populations: clusters 1 and 2, as well as population R37, which was intermediate between these two clusters. At HL3, we carried out the tests within each one of the clusters 1–4. Again, we treated intermediate populations as additional reference populations. For instance, within cluster 1, there were three reference populations: 'I', 'II' and R16, which was intermediate between 'I' and 'II' (see Fig. 2). In addition, we defined an additional hierarchical level (HL4) where single populations within each one of the 12 clusters I-XII from HL3 were considered as separate reference samples. Finally, we performed the analysis with all 38 populations from our North American reference dataset, considering no hierarchical subdivision.

For all GENECLASS2 computations, the total number of 13 nuSSRs needed to be reduced for the reference populations within the southern Rocky Mountain clusters XI and XII. Here, only 10 loci with reliable genotypes were available, because the loci 5A8, 3F1 and 2D6 revealed a high number of missing values. These loci contribute strongly to the differentiation to all other population clusters. The results of the self-assignment analysis are presented in Fig. 4.

Origin assignment of European Douglas-fir

The final step of our study was to "trace back" and identify the seed origin of the 67 European Douglas-fir stands listed in Table 1. We performed "assignment of groups of individuals" with the software GENECLASS2 (Piry et al. 2004). We applied the multilocus Bayesian criterion according to Rannala and Mountain (1997) with a threshold value of $p = 0.05$ for all runs. We carried out separate analysis at each hierarchical level. The reference populations for each analysis run followed the hierarchical order of the self-assignment test as shown in Fig. 3.

Prior to the assignment with GENECLASS2, we analyzed the individuals from the 67 European stands, together with the reference individuals (from the native range) from our 38 North-American reference stands, using the software STRUCTURE. We used a fixed $K = 2$ value to determine the assignment of each population and individual to one of the two varieties (coastal or Rocky Mountain). The purpose of this step was to detect cases of artificial intervarietal admixture in European stands. Next, we separated and removed pure Rocky Mountain Douglas-fir individuals ($Q < 0.1$) from introduced stands with affinity to coastal Douglas-fir ($Q \geq 0.9$) and vice-versa.

After removing these individuals, we performed successive assignment analyses with GENECLASS2 following the hierarchical structure of the reference populations. We assigned the European populations to one of the two

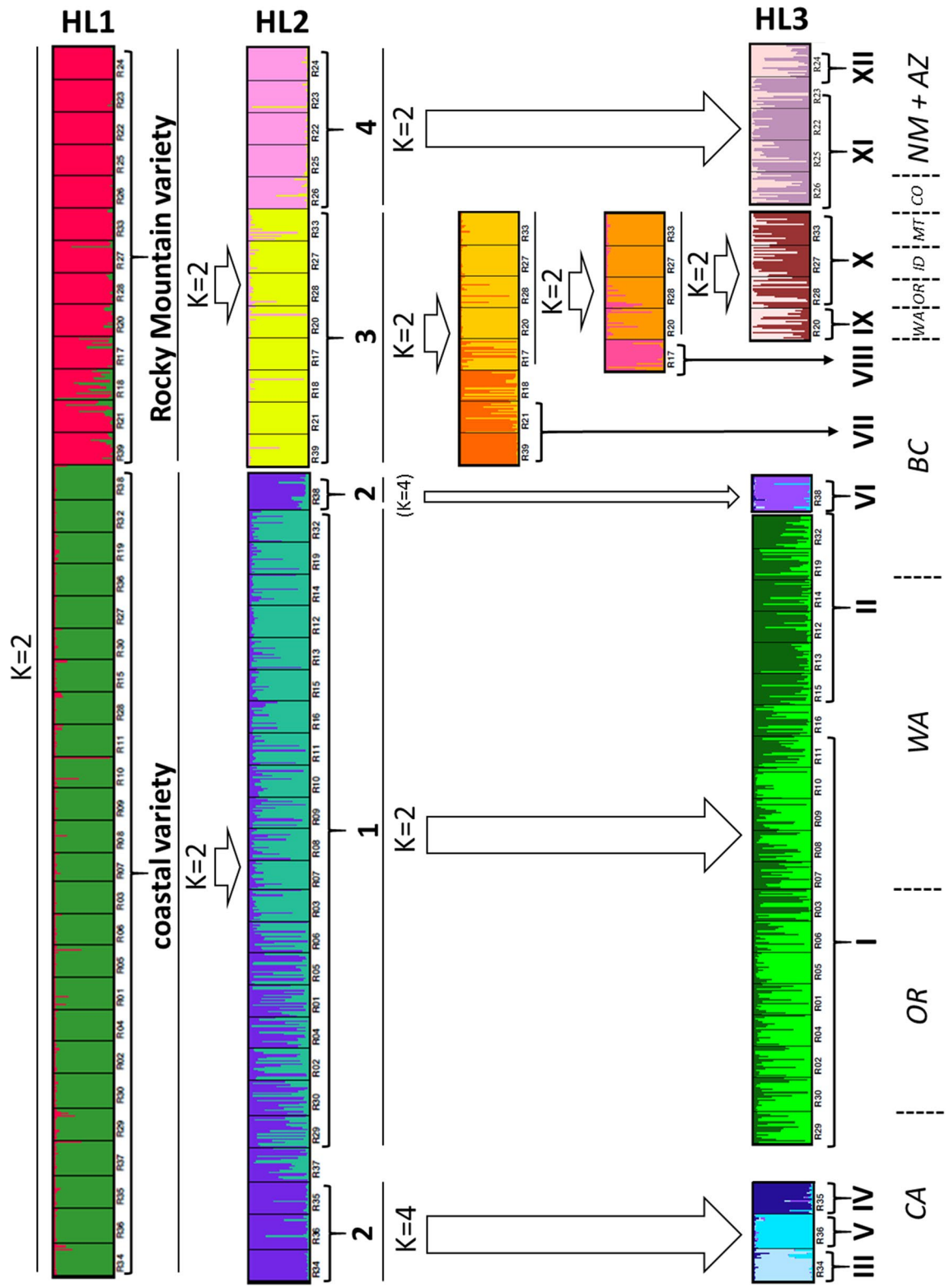

Fig. 2 Results of the hierarchical Bayesian cluster analysis of North American populations. Within bar plots, each derived subpopulation (cluster) is marked with a different color. Each population forms a box delimited by horizontal black lines (population designations R01–39). Each individual is represented with a horizontal bar. To the right of each bar plot, the resulting clusters at the corresponding hierarchical level (HL) are lettered (HL1: coastal variety and Rocky Mountain variety, HL2: 1–4, HL3: I–XII). Coastal Douglas-fir populations were sorted from south to north, Rocky Mountain populations from north to south. On the right-hand side, the state is marked where each population is located. *CA* California, *OR* Oregon, *WA* Washington, *BC* British Columbia, *ID* Idaho, *MT* Montana, *CO* Colorado, *NM* New Mexico, *AZ* Arizona

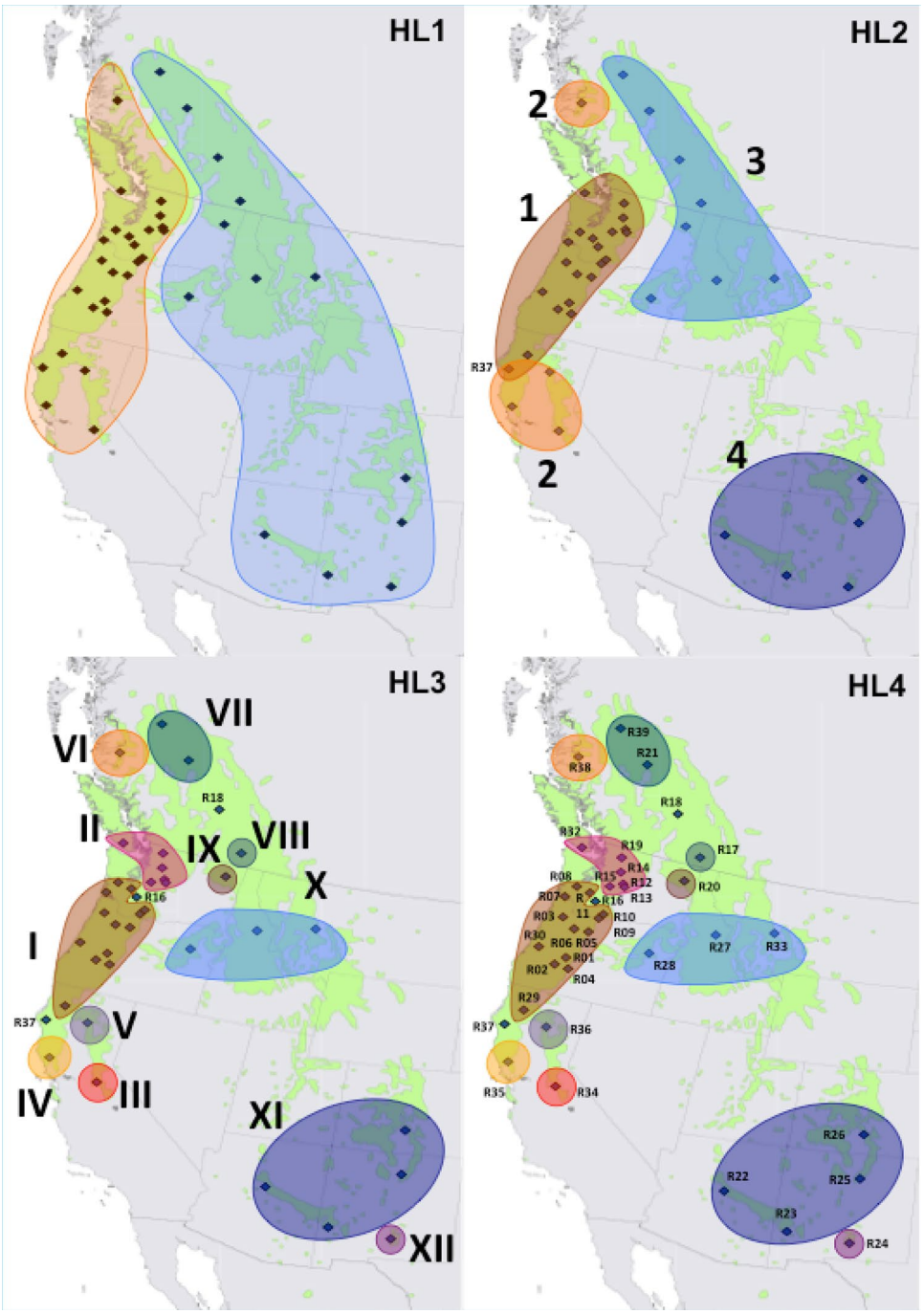

Fig. 3 Distribution map of the four hierarchical levels. Map HL1 shows the location of the coastal (red area) and Rocky Mountain (blue area) variety populations of the hierarchical level one. The four clusters with their corresponding populations of hierarchical level two are shown in map HL2. HL3 contains the 12 genetic clusters of hierarchical level three. HL4 shows the populations within the clusters of HL3 and are equitable to hierarchical level four. Three reference populations (R16, R18, R37) are cluster-admixed in this level

varieties at HL1. At this step, we used all 67 European Douglas-fir populations and assigned them to one of the two varieties. Next, we performed separate runs at the HL2 level within each of the two varieties. We employed the analyses at HL3 level, i.e. within each one of the clusters 1–4. The final step was to assign each of the 67 European populations to one of the 38 reference stands from North America by running the analysis within each one of clusters I–XII at HL4 according to the outline shown in Fig. 4.

The assignment tests resulted in a score for each assignment of a population to a given reference sample. This score ($score_{i,l}$) is defined as:

$$\text{Score}_{i,l} = \frac{L_{i,l}}{\sum L_{ij}}$$

where L_{ij} is the likelihood of an individual or a group of an individual i to belong to reference population l (Piry et al.

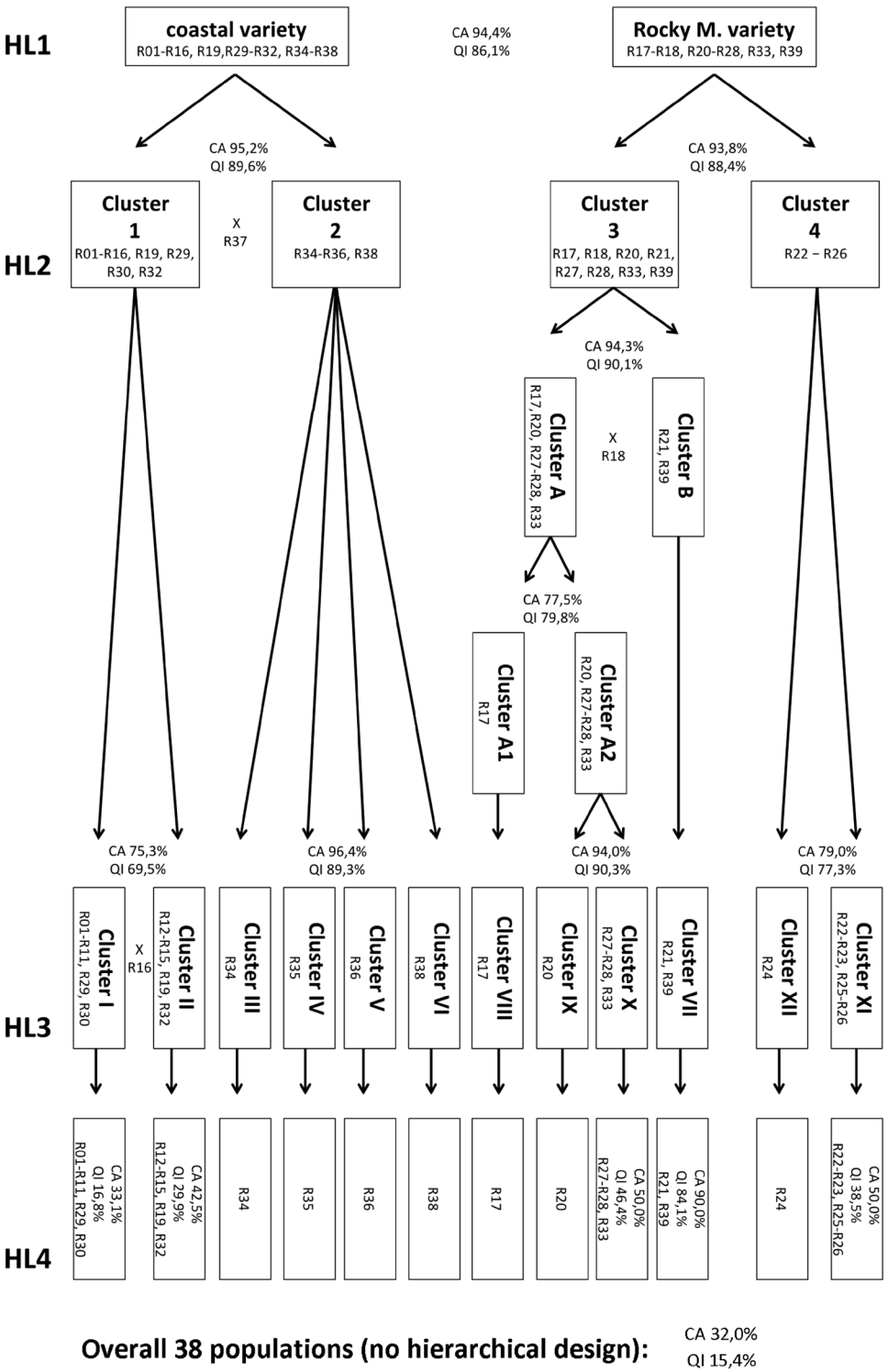

Fig. 4 Arrangement of the four hierarchical levels (HL1–HL4) starting from 38 populations of the reference data set grouped into the coastal and Rocky Mountain variety, followed by four and twelve genetic clusters. For each branch of the top-down procedure, the Quality index (QI) and the correctly assigned individuals (CA) were calculated with the self-assignment test in GENECLASS2. Populations marked with an X between two clusters are indicated as cluster admixed populations

2004). The summary of the assignment analysis results at the population level are shown in Table 2. The result details for each of our 67 European Douglas-fir stands of unknown origin are presented in the Appendix (Online Resource 3). 64 of the 67 European populations (see Table 2) come from areas located in Washington, Oregon and Northern California (clusters I and II). Figure 5 shows the assigned location of the 64 European Douglas-fir stands to their closest reference population in North America. Next, we performed additional assignment analysis at the individual level to identify possible cluster-admixed stands. Cluster-admixed stands are

Table 2 Assignment results by GeneClass2 for HL1–HL4. The clusters are arranged according to Fig. 2 N_{Eur} is the number of European Douglas fir stands assigned to the corresponding cluster

HL1	HL2	HL3	HL4	N_{Eur}
Coastal variety	1	I	R01	7
			R04	1
			R05	2
			R09	1
			R11	40
			R29	2
			R30	9
		II	R14	1
			R15	1
Rocky Mountain variety	3	Intermediate between VII and VIII, no HL4 assignment		2
	4	XII	R25	1

introduced populations which might have been established with more than one seed source.

Performing only the assignment for groups of individuals, we can assign a population of unknown origin to one of the reference populations. Hence, a genetically intermediate population (cluster-admixed) population cannot be identified. For instance, at HL1, there are only two reference populations corresponding to the two varieties. Thus, it is not possible to characterize a European population as intermediate when performing 'assignment of groups of individuals' using GENECLASS2, because no such reference population exists. Thus, we additionally performed "assignment of individuals" and calculated the proportion of assigned individuals per cluster.

Assuming that no admixture is evident, the number of individuals assigned to the 'true' cluster will depend only on the accuracy of the method and will thus be similar to the CA value from the self-assignment (the measure of method accuracy). Deviations between the two values suggest an intermediate origin. We defined a tolerance level of 10% in the deviation from the CA score. Any higher deviation indicates that cluster-admixed stands may exist. The results of the assignment analysis at the individual level for identifying possible cluster-admixed populations are presented for each of our 67 European Douglas-fir stands in Online Resource 4.

Final results and discussion

Using the reference data (see Fig. 1) which covers the full natural distribution range of Douglas-fir in North-Western America, we are able to "trace back" and identify Central European Douglas-fir stands planted at the end of the 19th and early twentieth century of unknown seed origin (see Table 1). Note that compared to the provenance trials which were established during the 1960s across Europe (Chakraborty et al. 2015) the seed origin of the old European Douglas-fir stands were unknown, although some of these stands are used as European Douglas-fir seed sources.

At the hierarchical level 1 (HL1), we assigned only one population (S05) to the Rocky Mountain variety by both STRUCTURE and GENECLASS2 (see Online Resource 3). Further two populations (AZ06 and HO4) were assigned to the Rocky Mountain variety using GENECLASS2, but showed mixtures with the coastal variety based on the results of the Bayesian clustering analysis (see Online Resource 3). The seed sources of the remaining 64 of our 67 investigated Douglas-fir stands come from cluster I and II within the hierarchical level 3 (HL3) which refer to locations in Washington, Oregon and northern California (Table 2). Figure 5 shows a detailed map with the European Douglas-fir seed stands and their identified North American seed source location.

In Germany and Austria, many recommended provenances (see also the provenance trials established in the 1960) originate from cluster I (coastal variety), which covers an area between central Washington and the northern part of California (Konnert et al. 2008; Weißenbacher 2008). Most of our investigated old Douglas-fir stands of unknown origin came from the same area covering population R11 (Ashford/Elbe, Washington) with more than half of our 67 analysed stands (see Table 2), followed by R30 (Zone 061, Oregon) (see Table 2). Two reasons for this result seem to be possible: (1) a selection of former planted stands from unsuitable provenances, where only the "adapted" provenances (i.e. from the area of cluster I) survived; (2) a selection of stands with some early knowledge using seeds form cluster I. For example, reports about the excellent performance since more than a century are evident (see Schwappach 1911; Kanzow 1937) and our results confirm that the detected origin of our old Central European Douglas-fir stands are consistent the recommendations (Konnert et al. 2008; Weißenbacher 2008).

If Douglas fir stands regenerate naturally, the information about the genetic admixture enhances silvicultural decision making (Eckhart et al. 2017). For instance, stands from unsuitable provenances will contribute to the genepool of the regeneration of suitable stands because pollinations have a negative effect on the regeneration (Konnert and Ruetz 2006).

From our analysis, two important facts are of interest: (i) several stands originated from areas outside the recommended regions (e.g. two stands were assigned to a reference population from northern California), indicating that additional seed sources may be suitable for Douglas-fir in Europe and (ii) the distribution of the identified seed origins

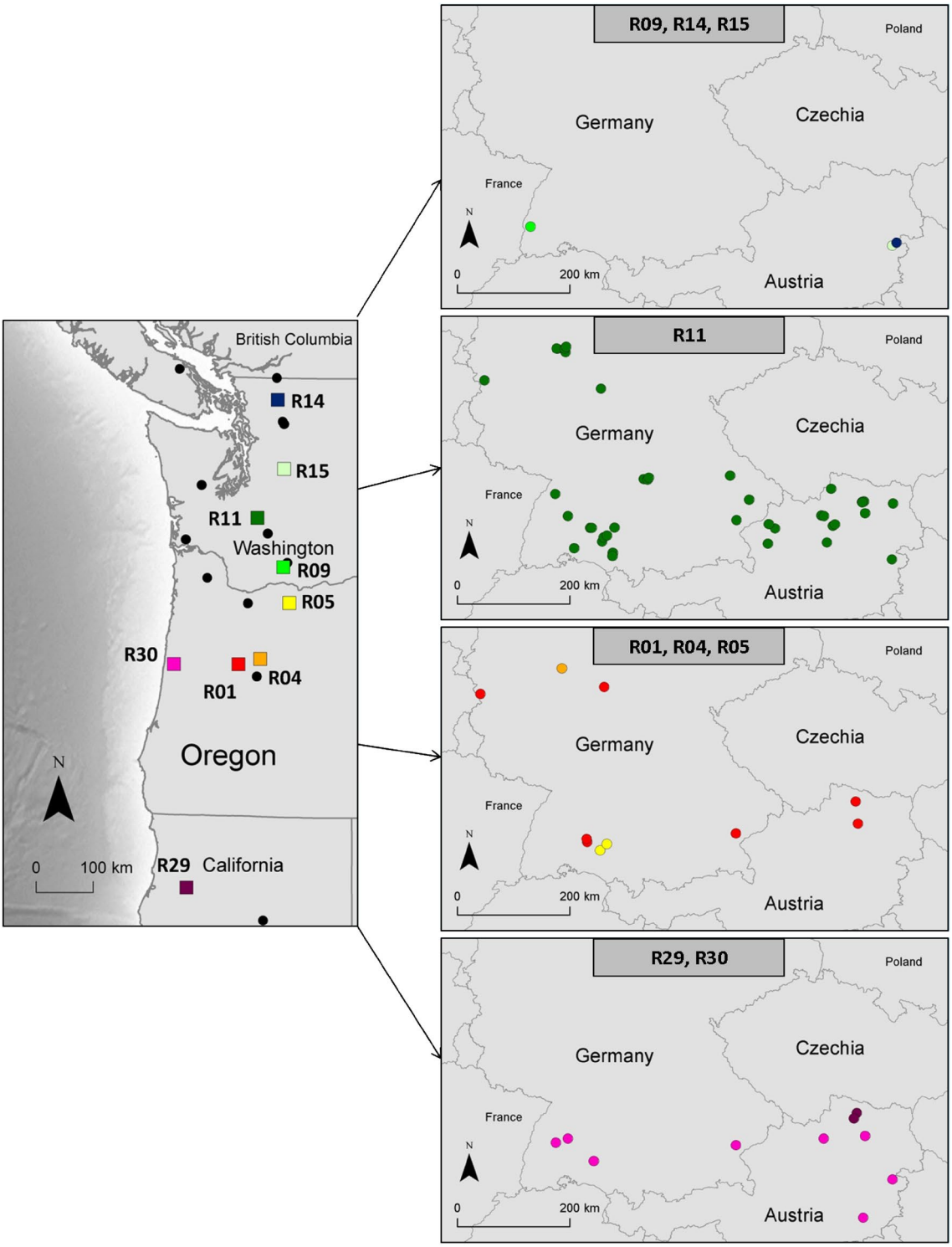

Fig. 5 Population assignment of European stands of coastal Douglas-fir. Left, the reference populations of origin are marked with squares filled with different colors. Black dots symbolize reference populations without any assigned European populations. Right, the European populations are sorted according to the assignment. The colors correspond to the reference populations from North America

of our 67 Douglas-fir stands did not follow any geographic or climatic pattern within Europe.

Stands of the same origin occur for example in western Germany as well as in eastern Austria, climatically very distinct geographic zones. This suggests that the establishment of these stands did not follow a systematic pattern. It is probably a result of the high genetic diversity of populations within cluster I and II (van Loo et al. 2015) and the resulting adaptation potential. This indicates the important role of genetic diversity of seed sources for Douglas-fir management.

As expected, the geographic distribution of the clusters (see Fig. 3) follows a similar pattern as shown by van Loo et al. (2015). Only minor differences are evident due to the stochasticity of the applied Bayesian clustering method (Gilbert et al. 2012). Most of the 12 clusters at the HL3 are genetically and spatially delineated (see Figs. 2, 3, Online Resource 2). For instance, in Cluster 2 all four populations were assigned to one separate cluster (clusters III, IV, V and VI) at a membership proportion greater 0.9 ($Q > 0.9$; see Fig. 2).

Within HL3, cluster I and II exhibit a significant differentiation (see Online Resource 2) with a varying gradient in the membership proportion (Q-values). In Cluster I, the membership proportion decreased from south to north, while in Cluster II, it increased from south to north (see Fig. 2). Similar effects in densely sampled areas have been reported in other studies (e.g. Ekins et al. 2006) and may be seen as an effect of isolation by distance. We addressed this problem by introducing intermediate populations ($0.4 < Q < 0.6$) e.g. R16 (Reference population 16) as separate reference populations in our assignment performance (see Figs. 2, 3, 4). This setting also influences the test for detecting cluster-admixed stands: while at the HL1 and HL2, only seven potentially cluster-admixed stands were identified, at HL3 16 potentially admixed Douglas-fir stands between the clusters I and II (see Online Resource 4) were registered. This suggests that results have to be interpreted carefully when clusters represent areas located in the mating contact zone.

Cluster I and II are located within the most important area of seed sources for Central Europe (Konnert et al. 2008; Weißenbacher 2008) and would not be detected without performing the hierarchical step by step analysis of the reference dataset (see Figs. 2, 3). This method is a powerful approach to analyse populations with varying sampling density among clusters or hierarchical phylogenetic structure within clusters (Neophytou 2014; Janes et al. 2017; Wang 2017).

While the forest areas in Oregon and Washington were intensively sampled, we only had a few reference populations from the North (British Columbia) and the South (California) in our reference dataset (see Fig. 1). In such a situation, there is a tendency to assign individuals to a common cluster even if they are phylogenetically different (Kalinowski 2011; Neophytou 2014). Indeed, pairwise F_{ST}s between British Columbian and Californian populations are high (see Online Resource 2) and do not support any common origin. Following our hierarchical approach, we were able to subdivide these two regions at the hierarchical level 3 (HL3; see Figs. 2, 3).

The accuracy assessment of our developed clustering approach based on a "leave-one-out-approach" method (see Fig. 4) revealed the highest consistency at the HL1 and HL2 (Hierarchal Level 1 and 2) with > 93.8% correctly assigned (CA) individuals to their clusters (variety) and a quality index (QI) of > 86.1% (see Fig. 4). At HL3 (Hierarchical Level 3), the clusters 2 and 3 (see Fig. 2), exhibit a CA of > 94% which strongly supports that seeds from Douglas-fir populations in California (clusters III, IV and V) or from British Columbia (cluster VI) are correctly classified. At the same Hierarchical Level, the clusters 1 and 4 exhibit a correct assignment (CA) between 75.3 and 79% and QI (Quality index ranging between 68.9 and 78.8% (see Fig. 2). This suggests that if Douglas-fir stands originate either from (i) the area including the northern Cascades and the southern part of Vancouver Island or (ii) the area between Southern Washington and Northern California, they have a 75.3% chance to be classified correctly. This is a result of (i) the low level of genetic differentiation and (ii) isolation by distance, which makes clustering difficult, as confirmed in previous findings with other tree species (Jolivet and Degen 2012; Degen et al. 2013; Nazareno and Dos Reis 2014).

At the lowermost hierarchical level, i.e. among single populations within a cluster from HL3, uncertainty of assignment increased. The best proportion of correctly assigned individuals (CA) was evident for cluster VII with 90%, while CA did not exceed 51.8% within clusters II, X and XI. Within the largest cluster I, only 33.1% of all individuals were assigned to their own population (see Fig. 2).

In addition to genetic differentiation, further factors affecting the accuracy of assignment are: (i) the number of analysed loci (in our study we used 13 high variable microsatellite loci; see Online Resource 1), (ii) their diagnostic power and (iii) the size of the reference populations (Cornuet et al. 1999; Bjørnstad and Røed 2002; Wang et al. 2009).

Conclusion

The selection of tree species, but also the most suitable provenance, is one of the key silvicultural decisions. Douglas-fir is considered as an important adaptation option for climate change because Douglas-fir exhibits a lower vulnerability if longer drought periods during the summer months occur versus our main native European tree species, e.g. Norway spruce or common beech. With an increase in temperature

and changes in precipitation pattern (longer drought periods during the summer months) our native tree species are increasingly vulnerable in some regions, since they require a continuous water supply (e.g. rain) during the growth period. Our results are important for Douglas-fir management, because the investigated stands (i) have survived a full rotation length, (ii) show an excellent growth performance, (iii) regenerate naturally and some are (iv) identified and used as basic material for the production of reproductive material under the selected category. In addition, our assignment method can be used for other important questions associated to a changing climate such as (1) where do other European Douglas-fir stands come from? (2) Do all European stands come from a distinct area of North America or is the source geographically scattered? (3) Do established European stands follow the national provenance recommendation or do outliers exist? (4) Is there a correlation between side conditions within Europe and the origin of old Douglas-fir stands or not? (5) Do distinct provenances grow on special side conditions?

Acknowledgements Open access funding provided by Austrian Science Fund (FWF). For financial support, we want to thank the following institutions, enterprises, state and private landowners: the Austrian Science Fund (FWF) (Project-ID: P26504), the Austrian Research Promotion Agency (FFG), the alps GmbH (Project: B04 AdaptAF B), the Austrian Chamber of Agriculture, the Chamber of Agriculture of Lower Austria, Austrian Federal Forests, Land and Forstbetriebe Niederoesterreich, the Metternich'sche Forstamt Grafenegg, the Forstamt Stift Goettweig, the Esterhazy Betriebe GmbH, the LIECO GmbH, the Montecuccolli Gut Mitterau, the Fuerst Star- hemberg'sche Familienstiftung, the Gutsverwaltung Bubna-Litic, the Hatschek Forste, the Hoyos'sche Forstverwaltung Horn, the Forstamt Ottenstein for financial support. Also, we would like to thank Lambert Weissenbacher from the Federal Research and Training Centre for Forests, Natural Hazards and Landscape for his help with the sample collection.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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9.3 Publikation III

Eckart T, Hintsteiner W, Lair G, Van Loo M, Hasenauer H (2014) The impact of Soil Conditions on the Growth of Douglas-fir in Austria. Austrian Journal of Forest Science 131(2):107-128

131. Jahrgang (2014), Heft 2, S. 107–128

Austrian Journal of Forest Science
Centralblatt für das gesamte Forstwesen

The Impact of Soil Conditons on the Growth of Douglas-fir in Austria

Der Einfluss des Bodens auf das Wachstum von Douglasie in Österreich

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Keywords: Douglas-fir, Climate change adaptation, Stand basal area increment model

Schlagwörter: Douglasie, Klimawandelanpassung, Bestandeskreisflächenzuwachs

Abstract

The Douglas-fir, native to western North America, is a drought-tolerant species and considered as one of the most promising key tree species in Western and Central Europe for forest adaption under changing climate conditions. The wide native distribution range of the Douglas-fir, covering a large latitudinal and elevation range, constitutes genetically differentiated populations. Thus, the selection of suitable proveniences and site conditions are of major importance in guaranteeing a successful cultivation outside its natural distribution range. In this study, we investigate how environmental conditions may influence the growth of Douglas-fir in Austria and Southern Bavaria-fir stands. We develop a basal area increment model based on stand density, tree size and site conditions. Furthermore, the environmental factors climate, topography and soil and their relationship versus site index

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are investigated. Eleven Douglas-fir stands from Austria and Bavaria are randomly selected. The genetic seed sources are from the Western Cascades in Washington and Oregon. The 10 year basal area increment per hectare (BAI10) was modeled with a nonlinear power function explaining 77 % of the existing variation.

Zusammenfassung

Die trockenresistente Douglasie ist eine nordwestamerikanische Baumart und gilt aufgrund der erwarteten Klimaerwärmung als eine der aussichtsreichsten Alternativbaumarten in West- und Mitteleuropa. Das große natürliche Verbreitungsgebiet umfasst unterschiedlichste Standorte und Klimabedingungen, an die sich die Douglasie regional angepasst hat. Aufgrund dieser hohen genetischen Differenzierung ist die Auswahl der geeigneten Herkunft sowie die richtige Standortswahl entscheidend für den Anbauerfolg der Douglasie außerhalb ihres Ursprungsgebietes. In dieser Studie wird der Einfluss des Standortes auf das Wachstum von Douglasienbeständen untersucht. Dazu wird ein Kreisflächenzuwachsmodell auf Bestandesebene mit den Eingangsparametern Bestandesdichte, Baumdimension und Standortsbedingungen entwickelt. Außerdem wird der Einfluss der Umweltfaktoren Klima, Topographie und Boden auf die Oberhöhenbonität untersucht. Die Studie basiert auf elf zufällig ausgewählten Douglasienbeständen in Österreich und Bayern mit bekannter Herkunft. Die untersuchten Douglasienbestände stammen aus dem Gebiet westlich der Kaskaden in Washington und Oregon. Der 10-jährige Kreisflächenzuwachs pro Hektar (BAI10) wurde mit einer nichtlinearen Potenzfunktion modelliert welche 77 % der Gesamtvariation erklärt.

1. Introduction

The range of European forests is mainly determined by climate, whereas climate extreme values limit the distribution of tree species rather than average values. Due to present and future changes of the major limiting factors temperature and precipitation, possible impacts of these changes are of interest. Climate change impacts on European forests vary regionally with diverse effects (Maracchi et al., 2005). While increasing temperature generally leads to higher photosynthesis activity, higher temperatures also trigger drought periods which are assumed to be limiting factors for tree growth in the future (Kapeller et al., 2012). In temperate forests, drought is a major constraint for forest stability and productivity. Due to the predicted increase in the frequency and duration of drought periods under a changing climate, this problem might become even more severe (Peters et al., 2013). Especially the Norway spruce, the most productive coniferous

species, is more susceptible to dry periods and may (very likely) not survive under future conditions in some parts of Europe (Kapeller et al., 2012). Hence, adaption strategies have to be developed, and plantations of more tolerant species are highly discussed in Europe. The Douglas-fir (Pseudotsuga menziesii), native to western North America, is a drought-tolerant species and considered as one of the most promising key tree species in Western and Central Europe for forest adaption under changing climate conditions. Moreover, high productivity rates, high wood quality, as well as suitable ecological characteristics like high stability constitute great expectations from Douglas-fir cultivations in Austria and elsewhere (Englisch, 2008, Kleinschmit and Bastien, 1992).

The wide native distribution range of the Douglas-fir (Little, 1971) (Fig. 2), covering a large latitudinal and elevation range, constitutes geneticall-ly differentiated populations (Kleinschmit and Bastien, 1992). Accordingly, the selection of suitable proveniences and site conditions are of major importance to guarantee a successful cultivation outside its natural distribution range (Englisch, 2008). The purpose of this work is to understand the growth of the non-native tree species, explained by these three factors: (i) stand competition, (ii) tree size and (iii) site quality. Furthermore, the influence of environmental factors on the site quality is investigated. A common indicator for site quality is the site index, defined as the mean dominant tree height at a given reference age (Kindermann and Hasenauer, 2005).

The objectives of this study are (i) to model basal area increment per hectare by a nonlinear power function, (ii) to investigate the environmental factors affecting Douglas-fir growth, and based on these environmental factors, (ii) to assess soil, topographic and climate factors deriving from the site index.

2. Material and methods

2.1. Study area

In the present study, eleven old Douglas-fir stands were randomly selected in Eastern Austria and Lower Bavaria (Fig. 1) to investigate soil, climatic and topographic factors affecting Douglas-fir growth. The study area represents a variety of climatic environments, with mean annual precipitation ranging from 583 to 1608 mm and an annual temperature gradient between 6.5 and 10.3 °C. The elevation of the study center plots vary from 202 to 786 m above sea level and a slope inclination between 3 and 19 degrees (Table 1). The parent material was determined based on the soil map of Austria (Weber, 1997) and Germany (BGR, 2006) with various granites, gneiss and one limestone bedrock material.

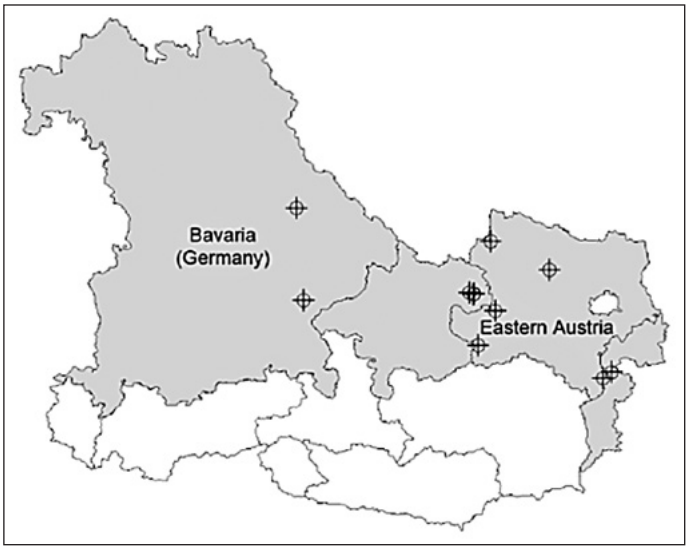


Fig. 1: Location of the study area and plots across Eastern Austria and Bavaria

2.2. Sampling design

All sample plots are located in pure even-aged Douglas-fir stands, with the following constraints:

(i) all stands belong to the coastal variety of the Western Cascades in Washington and Oregon, (ii) stand size ≥ 1 ha, (iii) proportion of Douglas-fir basal area ≥ 80 % and (iiii) a minimum age of 60 years.

The previously unknown origin of the selected Douglas-fir stands, which are recommended for seed harvesting and breeding, was assessed by genetic provenance analyses and could be assigned to the coastal variety of the Western Cascades in Washington and Oregon (Hintsteiner et al., 2014) (Fig. 2).

Fixed area plot

In each forest stand, a fixed area plot with a radius of 20 m (0.02 ha) was randomly selected to represent relatively uniform stand and soil characteristics. Forest stand, soil, and climatic characteristics were assessed on each plot through different variables, which are summarized in table 1.

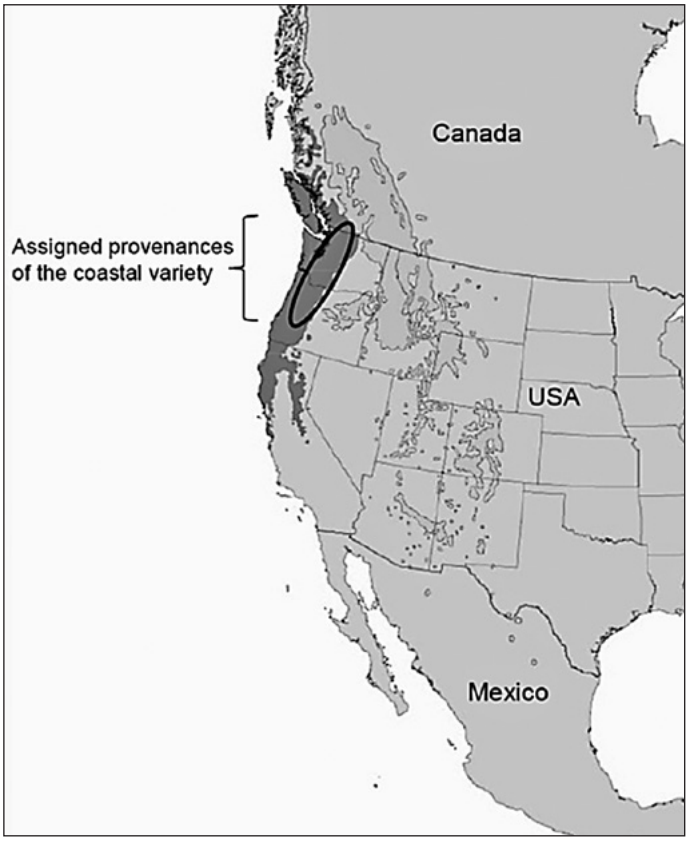


Fig. 2: Natural origin area of the coastal (dark grey) and inland (light grey) Douglas-fir in North America and assigned provenances of the Western Cascades in Washington and Oregon, (adapted after Little, 1971)

Within a radius of 20 m, the diameter at breast height (DBH), the horizontal distance and the azimuth to the plot center of each tree with a DBH ≥ 10 cm was measured. In order to calculate the stand age and the basal area increment, wood core samples at breast height were taken from five trees closest to the central point of the fixed area plot. Tree height and height to the living crown base were measured with a vertex.

Table 1: Variables and analytical procedures concerning forest stand, topography, soil and climate

	Variable		Unit	Mean (min-max)	Method
Stand parameters	DBH	Diameter at breast height	cm	62 (41 - 82)	Tree tape
	H	Height	m	40 (23 - 50)	Vertex
	ELEV	Elevation	m a.s.l	538 (202 - 786)	GPS
	SLO	Slope inclination	Degrees	12 (3 - 19)	Inclinometer
	Aspect	Aspect	Degrees	139 (6 - 337)	Wyssen compass
	rel. BAI10	Basal area increment 10yr	%	15 (7-21)	Tree ring analysis
	CCF	Crown competition factor	[-]	145 (40 – 210)	Krajicek et al. (1961), Hasenauer (1997)
	μDBH_1	Mean DBH at the beginning of the growing period	cm	54 (39 – 71)	Equation 6
	SI	Site index 100yr	m	46 (36 - 56)	Mitscherlich and Richards (1919), Kindermann and Hasenauer (2005)
Soil parameters	pH H2O*	Actual pH	[-]	4.5 (4.0 - 5.9)	ÖNORM L 1083
	pH CaCl2*	Potential pH	[-]	4.0 (3.7 - 5.4)	ÖNORM L 1083
	C*	Carbon	%	2.8 (1.4 - 5.4)	ÖNORM L 1080
	N*	Nitrogen	%	0.2 (0.1 - 0.3)	ÖNORM L 1082
	C/N*	C/N ratio	[-]	16.7 (12.6 - 21.0)	ÖNORM L 1082
	AVP*	Available phospor	kg/ha	32 (1 - 139)	Bray and Kurtz 1945
	Ca*	Calcium	kg/ha	4680 (249 - 25589)	ÖNORM L 1086-1
	Mg*	Magnesium	kg/ha	257 (19 - 1058)	ÖNORM L 1086-1
	K*	Potassium	kg/ha	110 (54 - 221)	ÖNORM L 1086-1
	Na*	Natrium	kg/ha	35 (9 - 122)	ÖNORM L 1086-1
	Al*	Aluminum	kg/ha	663 (263 - 1257)	ÖNORM L 1086-1
	Fe*	Iron	kg/ha	17 (0.7 - 52)	ÖNORM L 1086-1
	Mn*	Manganese	kg/ha	120 (18 - 305)	ÖNORM L 1086-1
	H+*	Hydron	mmol/kg	2.4 (0.5 - 4.3)	ÖNORM L 1086-1
	CEC eff.*	Cation-Exchange Capacity	mmol/kg	164 (61 - 654)	ÖNORM L 1086-1
	Bsat*	Base saturation	%	43 (13 - 91)	ÖNORM L 1086-1
	PO43-*	Phosphate	kg/ha	1 (0 - 4)	ÖNORM L 1092
	NO3-*	Nitrate	kg/ha	33 (1 - 77)	ÖNORM L 1092
	NO2-*	Nitrite	kg/ha	1 (0 - 13)	ÖNORM L 1092
	SO42-*	Sulfate	kg/ha	101 (41 - 248)	ÖNORM L 1092
	Clay*	Clay	%	17 (13 - 24)	ÖNORM L 1061
	Silt*	Silt	%	42 (30 - 64)	ÖNORM L 1061
	Sand*	Sand	%	40 (13 - 50)	ÖNORM L 1061
	Skeleton*	Skeleton	%	17 (1 - 34)	ÖNORM L 1061
	PV*	Pore volume	%	67 (55 - 78)	ÖNORM L 1068
Climatic parameters	A-PPT	Mean annual precipitation	mm	886 (583 - 1608)	DAYMET
	S-PPT	Mean summer precipitation [June, July, Aug]	mm	324 (234 - 551)	DAYMET
	W-PPT	Mean winter precipitation [Dec, Jan, Feb]	mm	160 (83 - 340)	DAYMET
	A-TEMP	Mean annual temperature	°C	8.2 (6.5 - 10.3)	DAYMET
	S-TEMP	Mean summer temperature [June, July, Aug]	°C	19.4 (15.4 - 17.1)	DAYMET
	W-TEMP	Mean winter temperature [Dec, Jan, Feb]	°C	-3.2 (-6.3 - 2.1)	DAYMET

*Separately assessed in A and B horizon (0-35 cm)

2.3. Stand parameters

Topography

On each sample plot topographic characteristics were assessed through different variables as listed in table 1. Elevation, slope inclination, aspect and coordinates of each forest stand were taken at the fixed area plot center and measured by a GPS device, inclinometer and Wyssen-compass, respectively.

Stand basal area increment

The basal area increment per hectare over past 10 years in percent was calculated as follows:

$$rel.BAI10 = \frac{rmsBAI10 * N * BUF}{BA} \quad [1]$$

$$BUF = \frac{10000}{A} \quad [2]$$

rel.BAI10 is the 10 year basal area increment in percent, rmsBAI10 is the quadratic mean of the 10 year basal increment of the sample trees, N is the number of Douglas-fir trees, BUF is the blow up factor, BA is the actual basal area per hectare. To get values per hectare, the blow up factor was calculated by dividing 10000 (1 ha) through the area of the plot (A, m²).

Index of stand density

Stand density of forests is commonly assessed with the crown competition factor (CCF) or the stand density index (SDI) (see Hasenauer et al., 2012). For this study, the CCF was applied (Monserud and Sterba, 1995). According to Krajicek et al. (1961), the CCF is the sum of the species-specific potential crown area (PCAi) divided by the plot area (A).

$$CCF = \frac{\sum PCA_i}{A} \quad [3]$$

The potential crown area was calculated with the open grown crown widths (CW, in m) given by Hasenauer et al. (1997), which defines the crown area of a tree at a given diameter at breast height (DBH, in cm) assuming open grown conditions.

$$PCA = \frac{\pi * CW^2}{4} \quad [4]$$

$$\ln(CW) = a + b * \ln(dbh) \quad [5]$$

a and b are species specific coefficients (table 2)

Table 2: Species specific coefficients for the crown width function after Hasenauer (1997).

Tree species	a	b
Norway spruce and other coniferous trees	-0.323	0.6441
Larch	-0.339	0.6823
Black pine	-0.157	0.631
Beech and other broad-leafed trees	0.2662	0.6072

Tree size effect

To describe the mean diameter increment relative to the tree size, the mean diameter at breast height at the beginning of the growing period (µDBH1) was calculated as follows:

$$\mu DBH_1 = \mu DBH_2 - \mu id_{10} \quad [6]$$

μDBH_2 is the arithmetic mean diameter at breast height of the sample trees at the end of growing period and μid_{10} is the mean 10 year diameter increment.

Index of site quality

The site index, as the most common indicator of site quality, is defined as the mean dominant tree height at a given reference age (Kindermann and

Hasenauer, 2005). In this study, SI was defined as the mean height of the 100 tallest trees per hectare (2 tallest trees on 0.02 ha plot) at the base age of 100 years. To calculate the site index at reference age, tree growth functions available for different tree species can be used by applying the dominant height growth function after Mitscherlich/Richard (1919) for the "Douglas-fir northwestern Germany", the SI_{100} was iteratively calculated:

$$OH = a(1 - e^{-b*t})^{c} \quad [7]$$

$$a = a_0 + a_1 * OH_{100} + a_2 * OH^2{}_{100}$$

$$a = a_0 + a_1 * OH_{100} + a_2 * OH^2{}_{100}$$

$$c = c_0 + c_1 * OH_{100} + c_2 * OH^2{}_{100}$$

OH = dominant tree height

OH_{100} = dominant tree height at the age of 100 (directly correlated to SI)

t = stand age at DBH + 10 years

a, b, c = coefficients for „Douglas-fir northwestern Germany" based on the yield table after Bergel (1985) from table 3

Table 3: Coefficients for the dominant tree height function after Mitscherlich/Richard (1919) for "Douglas-fir northwestern Germany (DoNwd)" (Kindermann and Hasenauer, 2005)

	a0	a1	a2	b0	b1	b2	c0	c1	c2
DoNwd	-5.92E+00	1.26E+00	0	9.90E-02	-2.79E-03	2.54E-05	9.43E+00	-3.15E-01	3.03E-03

2.4. Soil parameters

The soil characteristics were assessed through borehole samples and separated into A and B horizon (0-35 cm). In total, 44 borehole samples of four transects distributed across each forest stand were taken in order to overcome the expected heterogeneity of forest soils. The chemical and physical

soil properties were analyzed for each transect by standard laboratory methods. Four density core samples per forest stand were taken at the center of each transect to determine the pore volume of A and B horizon. The analytical results were expressed as soil stocks on a mass per unit area basis using bulk density, coarse fragment content, and depth of A and B horizon (0-35 cm). The soil variables listed in table 1 are the weighted mean values of A and B horizon.

2.5. Climatic parameters

The Austrian version of the climate interpolation model DAYMET was applied to calculate daily climatic data (minimum and maximum temperature and precipitation as well as solar radiation) for each study plot from 1960 to 2010 (Thornton et al., 1997, 2000, Hasenauer et al., 2003).

3. Statistical analysis

3.1. Stand basal area increment model

Stand basal area increment per hectare is determined by the three factors: (i) stand competition, (ii) tree size and (iii) site index parameters (Wykoff, 1990). Thus, a nonlinear power function including these three factors was defined:

$$rel.BAI_{10} = a * x_{SD}^{b} * x_{TSE}^{c} * x_{SI}\ d \qquad [8]$$

rel. BAI is the 10 year relative basal area increment per hectare of the Douglas-fir stands, x_{SD}, x_{TSE}, x_{SI} are variables corresponding to the three factors stand density, mean tree size and site index, and a, b, c, d represent model coefficients. The coefficients b and d describe the curvature of the power function. The parameterization of the nonlinear model function rel. BAI_{10} was carried out with the statistical program PASW 18. In order to test for multicollinearity, the variation inflation factor (VIF) after Snee (1973, 1977) was calculated for each explanatory variable.

$$VIF = \frac{1}{1-R^2} \qquad [9]$$

A large VIF (e.g. > 5) would indicate a strong correlation between certain predictor variables which can produce misleading results due to inadequate

independent information (Hasenauer, 1997).

3.2. Site index versus site variables

Linear regression plots of Site Index (SI) versus site variables did not indicate any non-linear relation. Thus the following multiple linear equation was used to study the variation of site index in relation to site variables:

$$SI = a + bx_t + cx_s + dx_c + \varepsilon \quad [10]$$

SI is the site index, x_t, x_s, x_c are variables corresponding to topographic, soil and climatic factors, a, b, c, d represent model coefficients and Ɛ is the additive error term. For statistical calculations, the program R was applied.

The analysis of the data followed four steps:

I. The selection of environmental factors that are significantly related to the variation in the SI and ecologically sound
II. The entering of significant variables sequentially according to their coefficient of determination R^2, starting with the most significant variable
III. Testing for multicollinearity according to a variation inflation factor (VIF) < 5 for each predictor variable after Snee (1973, 1977)
IV. The selection of the best model to explain and predict the SI of environmental variables according to the adjusted coefficient of determination $Radj^2$

4. Results

4.1. Prediction model for stand basal area increment

A basal area increment model at stand level was developed for the 11 Douglas-fir sites. The following nonlinear model was determined to predict the relative 10 year basal area increment projection per hectare for the Douglas-fir ($rel.BAI_{10}$):

$$rel.BAI_{10} = a * CCF^b * \mu DBH_1^c * SI * d \quad [11]$$

CCF is the crown competition factor, μDBH_1 corresponds to the mean diameter at breast height at the beginning of the growing period and SI is the

site index.

Due to the rather small sample size of only eleven Doulgas-fir stands, a resampling of the data was necessary to increase the accuracy of the model, defined by the standard error. The bootstrapping method was applied, as recommended by Adèr et al. (2008), to resample the original data set to a random sample size of 100 and to calculate the bootstrapped standard error. The resulting model explains 77 % of the variation in the 10 year basal area increment per hectare. All parameters entered the model significant at a 5 % probability level and multicolinearity was controlled to be a required variation inflation factor (VIF) < 5.

Table 4: Regression coefficients related to equation 11, the bootstrapped standard error of the nonlinear model and the coefficient of determination (R^2) as a measure of the goodness of fit.

	Coefficients	Estimates	Standard error*
Constant	a	8.587	13.284
CCF	b	-0.761	0.184
μDBH_1	c	-1.568	0.388
SI	d	8.591	13.281
R^2	0.77		
*Bootstrapped standard error based on a sample size of 100			

The predictor variables CCF and μDBH_1 correlate negatively and the SI positively, versus the predicted 10 year stand basal area increment per hectare.

Sensitivity analysis

The theoretical effects of the independent variables CCF, μDBH_1 and SI versus the stand basal area increment was tested by modeling the rel. BAI_{10} variation and carrying one variable within its measured range (minimum – maximum value) while the other independent drivers were kept constant at the corresponding mean values. Figure 3 provides the results for the competition factor CCF, which indicates the strongest effect, followed by the tree size factor μDBH_1 and the site index SI.

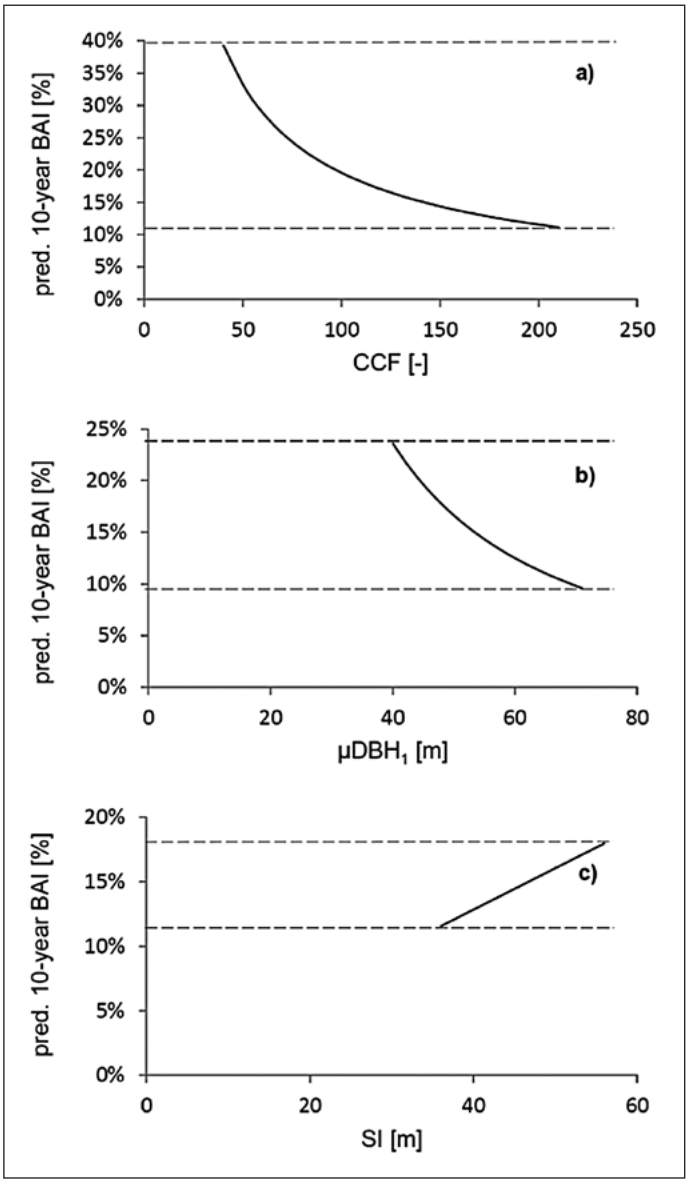
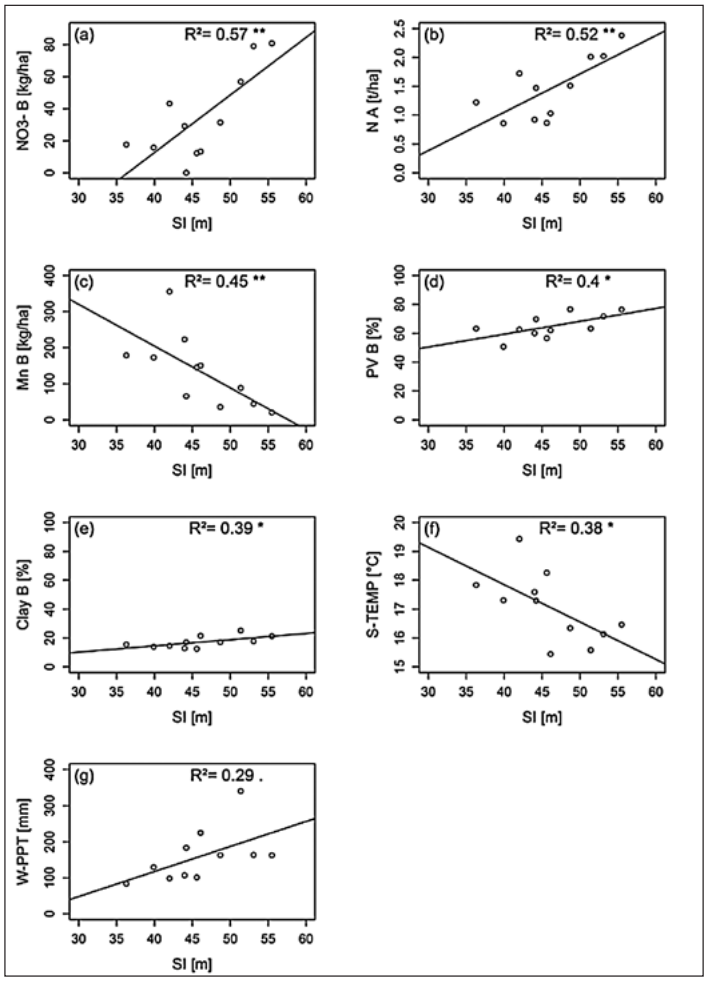


Fig. 3: Sensitivity analysis of the main influencing parameters CCF, μDBH_1 and SI
(a) CCF within the range of min – max and the mean values of SI and μDBH_1, (b) DBH_1 within the range of min – max and the mean values of CCF and SI (c) SI within the range of min – max and the mean values of CCF and μDBH_1

4.2. Site index versus site variables

The site index, defined as the dominant stand height attained at a particular age, may be considered as a surrogate of the environmental and/or site conditions for a forest stand. Thus, we next relate the site index to a number of soil parameters available for our forest sites to identify and explore the key drivers in the site index variation. Figure 4 provides the results including the correlation coefficients.



Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Fig. 4: Environmental factors (a-g) significantly related to the variation in Douglas-fir site index

According to Fig. 4, nitrogen in the form of nitrate (NO_3^-) in horizon B indicates the highest positive correlation and accounts for 57 % of the variation in SI. Manganese stock in horizon B (Mn B) is negatively correlated (R^2 0.45) to the SI. Pore volume PV and clay content in horizon B show positive correlations to the SI and account for 40 % and 39% of the variation, respectively. Mean summer temperature indicates a negative correlation to SI (R^2 0.38) and winter precipitation shows a weak positive correlation (R^2 0.29). In a next step, those variables with the greatest influence were sequentially combined into a multiple regression equation.

Best model output

Multiple linear regression method was used to calibrate a model for predicting the site index as it may depend on quantitative environmental drivers.

$$SI = a + bNO_3^- \text{ B} + cMn\ B \qquad [12]$$

The linear model includes the two soil variables NO_3^- B and Mn B, which are statistically highly significant at the 95 percent level, and explains 73 % percent of the variation in the site index (table 5). Multicolinarity was controlled by a requested inflation factor (VIF) of ≤ 5. The remaining standard error of the model estimate is 2.98 m.

Table 5: Estimates, standard error and p-values for the multiple regression model (equ. 12)

	Estimates	Standard error	p-value	VIF
Constant	45.501767	2.269763	< 0.001	
NO3- B	0.127458	0.036029	< 0.01	1.10
Mn B	-0.02842	0.009963	< 0.05	1.10
Radj²	0.73			

Sensitivity analysis

Again, the theoretical behavior of the predictor variables NO_3^- B and Mn B versus the resulting SI (Site index) was tested by modeling the SI develop-

ment for different levels by varying one parameter within the measured range (minimum to maximum value), while keeping the other parameters constant at its mean value (see Fig. 5). The effect of NO3- B and Mn B on the SI development ranges between 42 – 52 m and 40 – 49 m, respectively. According to Fig. 5, the predictor variables NO3- B and Mn B have a similar effect on the development of the site index.

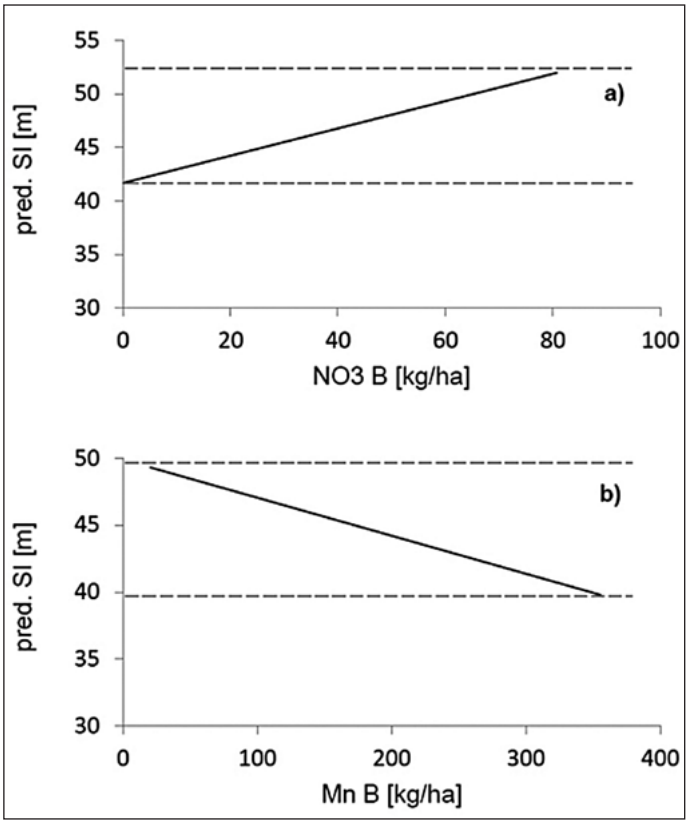


Fig. 5: Sensitivity analysis of the main influencing parameters NO3- B and Mn B (a) NO3- B within the range of min – max and the mean value of Mn B and (b) Mn B within the range of min – max and the mean value of NO_3^- B

5. Discussion

The stand level of the Douglas-fir model predicts the relative 10 year basal area increment per hectare as a nonlinear power function with the predic-

tor variables crown competition factor, mean diameter at breast height at the beginning of the growing period and site index (equ. 11). The basal area increment relative to tree size is greatest in low density stands with a high site quality. Theoretically, the accurate relationship between increment and tree size would have a positively skewed unimodal form as increment increases to a maximum in early life and then gradually decreases (Wykoff, 1990).

As our sampling design was restricted to forest stands with a minimum age of 60 years, no modification of the power function was necessary. The sensitivity analysis (Figure 3) indicated a substantial effect of the competition parameter CCF on the basal area increment. Note that in our data we had covered a wide range of CCF values (40-120) (see Table 1). For the tree size parameters, the sampling design restricted a broader range in our data due to the fact that we only recorded stands older than 60 years. This is also evident for the site conditions which would have required a broader range in the sampled forest stands.

The genetic material of our Douglas-fir stands comes from the coastal variety of the Western Cascades in Washington and Oregon (Hintsteiner et al., 2014). Furthermore we selected only pure even aged Douglas-fir forests by ensuring a relative Douglas-fir proportion in a total stand basal area of ≥ 80%. This suggests that any difference in the growth rates of our Douglas-fir stands are only driven by stand density as well as soil and/or site conditions. As shown in Table 4, crown competition factor CCF, a measure for stand density, mean diameter at breast height and the site index entered the model significantly.

A correlational study determining the key driver for the site index (see Figure 4 a-g) revealed that the large number of environmental parameters (Table 1) can be reduced to only seven where the two soil parameters NO_3^- B and Mn B explain about 73 % of the site index variation (see Table 5). While nitrogen is the most limiting macronutrient in forests all over the world (Littke Hanft, 2012), manganese as a micronutrient intervenes in photosynthesis (Millaleo et al., 2010). Previous studies that examined the correlations between nitrogen fertilization and growth response in the Pacific Northwest illustrated positive responses of Douglas-fir growth (e.g. Gardner, 1990; Miller et al., 1986). Moreover, nutrient deficiencies in Douglas-fir stands were consistently demonstrated only for nitrogen (Weetman et al., 1992), which is consistent with our results.

Compared to the positive effect of nitrogen, manganese was negatively correlated to the site index (Fig. 4c). Manganese is an essential micronu-

trient for plants but may turn toxic in excessive concentrations. The amount of available manganese is influenced by soil pH, excess of water, poor drainage or applications of organic material (Millaleo et al., 2010). According to our study, the negative impact of manganese could be an indication of waterlogged soil conditions, as Mn B is positively correlated to the pore volume in horizon B ($R^2 = 0.40^*$). No correlation between Mn B and soil pH B was found. The sensitivity analysis (Figure 5) showed an equal effect of both soil parameters on the Douglas-fir site index.

Pore volume PV, indicating the air- and water holding capacity of soils, and clay content, alluding the water- and nutrient holding capacity of soils, in horizon B showed positive correlations to the SI (Fig. 4 d-e). The positive effect of the soil parameter clay content in B horizon is in agreement with the results of the soil-site study after Steinbrenner (1979) on sedimentary and volcanic soils in western Oregon. Other studies also revealed negative correlations of clay content and site index (e.g. Corona et al., 1998). Soil nutrients and soil texture were varied highly within all assigned bedrock materials (gneiss, granite, limestone). Accordingly, the use of soil parent material differentiated site indices would not be meaningful.

Correlations between climatic parameters and the SI are evident, with a negative correlation of mean annual summer temperature (Fig. 4f) and a positive correlation of mean winter precipitation (Fig. 4g) versus the SI. Under a changing climate with projected increases of mean summer temperatures and mean winter precipitation in Austria (Loibl et al., 2011), the climate sensitivity of the Douglas-fir is of particular interest to enable a successful incorporation in adaptive forest management considerations.

No significant correlations were found between the SI and the remaining environmental parameters (see Table 1), possibly due to the limited range of the selected Douglas-fir sites. To enlarge the gradient of the environmental parameters, Douglas-fir stands developed under extreme conditions should be sampled. This includes Douglas-fir sites under dry conditions as well as calcareous sites. Current recommendations for Douglas-fir cultivation in Austria and Germany are basically restricted to non-calcareous soils (e.g. Englisch, 2008), which could not be confirmed in our study.

Acknowledgements

This research was conducted as part of the COMET research project "Investigation and Optimization of Forest Management in Austria under Cli-

mate Change Using the Example of Douglas Fir", granted by the Austrian research promotion agency (FFG) and the provinces Tyrol and Vorarlberg. We want to thank our project partners for their financal support: Landwirtschaftskammer Österreich, Landwirtschaftskammer Niederösterreich, Land und Forstbetriebe Niederösterreich, Metternich`sche Forstverwaltung Grafenegg, Forstamt Stift Göttweig, Esterhazy Betriebe GmbH, LIECO GmbH, Montecuccolli Gut Mitterau, Fürst Starhemberg`sche Familienstiftung, Gutsverwaltung Bubna-Litic, Hatschek Forste, Hoyos`sche Forstverwaltung Horn, Österreichische Bundesforste AG, Forstamt Ottenstein.

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