Fakultät für Forstwissenschaften und Waldökologie
Abteilung Forstgenetik und Forstpflanzenzüchtung

# Genetische Charakterisierung der slawonischen Stieleiche (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) in Deutschland

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**Tag der Disputation**:

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# Abkürzungsverzeichnis

| | |
|---|---|
| AFLPs | Amplified Fragment Length Polymorphism |
| $A_R$ | allelic richness |
| BLAST | Basic Local Alignment Search Tool |
| BMEL | Bundesministerium für Ernährung und Landwirtschaft |
| Bzw | beziehungsweise |
| cf. | confer, vergleiche |
| cM | centiMorgan |
| cp | Chloroplast |
| cpDNS/DNA | Chloroplasten- Desoxyribonukleinsäure/ deoxyribonucleic acid |
| cpSSR | chloroplast Simple Sequence Repeat |
| °C | Grad Celsius |
| ddRADseq | double-digested Restriction site-Associated DNA sequencing |
| DKV | deutsche Kontrollvereinigung |
| DNA/DNS | deoxyribonucleic acid/ Desoxyribonukleinsäure |
| e.g. | for example |
| EST-SSR | Expressed Sequence Tag-derived Simple Sequence Repeat markers |
| Fig. | figure |
| $F_{IS}$ | inbreeding coefficient |
| $F_{ST}$ | fixation Index |
| GBS | Generation by Sequencing |
| gSSR | genomic Simple Sequence Repeat |
| $H_e$ | expected heterozygosity |
| $H_o$ | observed heterozygosity |
| HP | haplotype, Haplotyp |
| IND | indigenous |
| LD | Linkage Disequilibrium |
| LG | Linkage Group |
| LGf | female Linkage Group |
| LGm | male Linkage Group |
| M ü.M. | Meter über Meeresspiegel |
| NGS | Next Generation Sequencing |
| NR | Natural Regeneration |
| NRW | Northrhine-Westfalia |
| nSSR | nuclear Simple Sequence Repeat |
| PCoA | Principal Coordinate Analysis |
| PCR | polymerase chain reaction |
| Q. | *Quercus* |
| Qrob | *Quercus robur* |
| QTL | Quantitative Trait Loci |
| RADseq | Restriction site-Associated DNA sequencing |
| RAPD | Random Amplification of Polymorphic DNA |
| RFLPs | Restriction Fragment Length Polymorphisms |
| SHK | Sonderherkunft |
| SLAV | Slavonian |
| SNP | Single Nucleotide Polymorphism |
| SSR | Simple Sequence Repeat, microsatellite |
| subsp. | Subspecies |
| u.a. | unter anderem |
| z.B. | zum Beispiel |

## Summary

The structure and composition of forests have a strong influence on forest ecosystems and their services and functions. From today's perspective, important properties such as ecosystem stability, biodiversity conservation and carbon storage capacity can be influenced by forest management. Actions toward forest restructuring are needed as response to the consequences of climate change, which includes the exploration of alternative tree species for forestry interest and thus becoming the focus of more research. Among them, the Slavonian pedunculate oak (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás), originated from eastern Croatia is of a particular interest due to its high growth performance and straightness. In Germany, the so far relatively unknown subspecies of pedunculate oak occupies a special position as a naturalized variety. However, it could help to improve the adaptability of future mixed oak forests, as its area of origin already shows climate data currently predicted for Germany in the future. In order to estimate the genetic potential of this tree species, worthwhile stands have to be investigated with regard to their genetic variability, structure and diversity. Through an extensive pool of genetic resources, the plants are enabled to adapt to changing environmental conditions, for example through gene flow. On the basis of the investigations carried out within the contextualized framework, this dissertation is intended to provide a valuable contribution to the field of forest genetics, focusing on:

The study (Chapter 2) titled "The Slavonian oak (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) as an Alternative for Climate Change: Experimental and Genomic Resources" deals with the research status of the Slavonian oak in Germany and establishes a fundamental basis for the subject matter. The main focus of this study is the investigation of the Slavonian oak with regard to its use as an alternative tree species for forest conversion in Germany, taking into account predicted climate changes. Combined with the low forestry interest of this variety, there are only few studies of genetic characterization, genetic variation and/or trait variation. This manuscript discusses relevant studies and results investigating adaptation-related and spatial genetic variation of Slavonian oak stands and associations with trait variation in an intraspecific crossing progeny [*Q. robur* subsp. *robur* x *Q. robur* subsp. *slavonica*].

Considering that the determination of the geographic origin determination of Slavonian oak populations in Germany has been predominantly studied using chloroplast DNA markers, the following manuscript presented in chapter 3 explores the genetic differentiation of Slavonian and indigenous oak populations in western Germany (North Rhine-Westphalia) using nuclear DNA markers. In this study, samples from 37 indigenous and Slavonian populations, which could already be characterized well in previous studies with chloroplast DNA markers,were analysed at 20 nuclear DNA markers. Genetic variation is considered an important prerequisite for sustainable use of genetic resources and

to provide long-term adaptability of tree species. Using the nuclear DNA marker set, populations with haplotypes naturally occurring in both Germany and Croatia could be well distinguished. Overall, the results show that both nuclear and chloroplast DNA markers are necessary to distinguish between genetically very similar taxa. The combination of both marker types allows the distinct identification of the pedunculate oak varieties in mixed stands and helps to certificate the origin of reproductive material.

In the study dealed in chapter 4, stands of indigenous and Slavonian oak and their natural regeneration were investigated with regard to their genetic variation and spatial structures. In Germany, in addition to artificial stand establishment, natural regeneration is a predominantly silvicultural practiced form of forest regeneration that allows the genetic composition to be conserved. Besides quantitatively sufficient self-differentiation and well distributed individuals in the regeneration layer, genetic variation and spatial genetic structures are relevant for the quality and vitality of the stand. For the fourth study, therefore, two Slavonian and two indigenous stands and their natural regeneration were genetically characterized at both 23 nuclear DNA markers and 5 chloroplast DNA markers. The results show that the genetic information of old-growth stands is almost completely inherited to the next generation. The reason for this is the similar, high level of genetic variation, low genetic differentiation, and similar to matching haplotype composition as exhibited by the respective old-growth stands. Since genetic diversity has a strong impact on the adaptability of populations to changing environmental conditions and their survival, high genetic variability can ensure stability and sustainability of forest ecosystems. Furthermore, there was no evidence of hybridization or gene flow between Slavonian and indigenous populations. A possible reason for this could be the different timing of bud burst of the studied taxa.

The last chapter of the dissertation (Chapter 5) deals with the construction of the first RADseq (restriction site-associated DNA sequencing) SNP marker-based, high-resolution, genetic linkage map (male and female) within the intraspecific *Quercus robur* - full sib family (*Q. robur* × *Q. robur* ssp. *slavonica*). For the construction of the female and male genetic linkage maps, 249 progenies of the controlled intraspecific cross *Q. robur* x *Q. robur* ssp. *slavonica* were used. Genetic linkage maps were generated using the *pseudo-testcross* method and the *Kosambi* mapping function. A total of 473 SNP markers were mapped on the female linkage map and 502 SNP markers were mapped on the male linkage map, each consisting of 12 linkage groups. These maps provide a valuable basis for conducting future quantitative trait loci (QTL) analyses, identification of genes involved in adaptive trait variation, and comparative genomic analyses.

# Zusammenfassung

Der Aufbau und die Bestockung von Wäldern haben einen starken Einfluss auf Waldökosysteme sowie deren Leistungen und Funktionen. Aus heutiger Sicht wichtige Eigenschaften wie Ökosystemstabilität, Erhalt biologischer Vielfalt und Kohlenstoffspeicherfähigkeit lassen sich über verschiedene Ansätze der Bewirtschaftung beeinflussen. Mit dem durch klimatische Veränderungen notwendig gewordenen Waldumbau gewinnen immer häufiger vorher kaum beachtete Alternativbaumarten an forstlichem Interesse und rücken damit stärker in den Fokus der Forschung. Eine dieser Alternativbaumarten ist die aus dem Osten Kroatiens stammende slawonische Stieleiche (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás). In Deutschland nimmt die bisher relativ unbekannte Art als eingebürgerte Varietät eine Sonderstellung ein. Sie könnte jedoch helfen die Anpassungsfähigkeit zukünftiger Eichenmischwälder zu verbessern, da ihr Ursprungsgebiet bereits gegenwärtig Klimadaten aufweist, die zukünftig für Deutschland prognostiziert werden. Um das genetische Potential dieser Baumart abschätzen zu können, müssen lohnenswerte Bestände hinsichtlich ihrer genetischen Vielfalt, Struktur und Diversität untersucht werden. Nur eine umfassende genetische Ausstattung ermöglicht es den Pflanzen sich u. a. über Genfluss, an veränderliche Umweltbedingungen anzupassen. Anhand der in den nachfolgend beschriebenen Studien durchgeführten Untersuchungen soll diese Dissertation einen Beitrag dazu leisten:

Die Studie (Kapitel 2) mit dem Titel „Die slawonische Stieleiche (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) als Alternative für den Klimawandel: Experimentelle und genomische Ressourcen“ befasst sich mit dem Forschungsstand der slawonischen Stieleiche in Deutschland und dient als Themeneinleitung. Den Schwerpunkt dieser Studie bildet die Untersuchung der slawonischen Stieleiche hinsichtlich einer Nutzung als Alternativbaumart für den Waldumbau in Deutschland unter Berücksichtigung prognostizierter Klimaveränderungen. Einhergehend mit dem bisher geringen forstlichen Interesse dieser Varietät, gibt es bisher nur wenige Untersuchungen der genetischen Charakterisierung, genetischen Variation und/oder Merkmalsvariation. In diesem Manuskript werden einschlägige Studien und Ergebnisse zur Untersuchung anpassungsrelevanter und räumlicher genetischer Variation slawonischer Stieleichenbestände sowie Assoziationen mit der Merkmalsvariation in einer innerartlichen Kreuzungsnachkommenschaft [*Q. robur* subsp. *robur* x *Q. robur* subsp. *slavonica*] erörtert.

Vor dem Hintergrund, dass die geographische Herkunftsermittlung slawonischer Stieleichenbestände in Deutschland bisher überwiegend anhand von Chloroplasten-DNA-Markern untersucht wurde, befasst sich das nächste Manuskript (Kapitel 3) mit der genetischen Differenzierung slawonischer und einheimischer Stieleichenbestände in Westdeutschland (Nordrhein-Westphalen) über Kern-DNA-Marker. Hier wurden Proben von 37 einheimischen und slawonischen Populationen,

die sich bereits in früheren Studien über Chloroplasten-DNA-Markern sehr gut charakterisieren ließen, an 20 Kern-DNA-Markern untersucht. Genetische Variation gilt als wichtige Voraussetzung für eine nachhaltige Nutzung genetischer Ressourcen und um eine langfristige Anpassungsfähigkeit der Baumarten zu gewährleisten. Unter Verwendung des Kern-DNA-Markersets ließen sich die Populationen mit Haplotypen, die sowohl in Deutschland als auch in Kroatien natürlich vorkommen, gut unterscheiden. Insgesamt zeigen die Ergebnisse, dass sowohl Kern- als auch Chloroplasten-DNA-Marker notwendig sind, um die genetisch ähnlichen Taxa zu unterscheiden. Die Kombination beider Markerarten lässt damit erstmals eine eindeutige Identifizierung der Stieleichenvarietäten in Mischbeständen zu und hilft innerhalb der Zertifizierung die Herkunft des Vermehrungsmaterials nachzuweisen.

In einer weiteren Studie (Kapitel 4) wurden Bestände aus einheimischer und slawonischer Stieleiche sowie deren Naturverjüngung hinsichtlich ihrer genetischen Variation und räumlichen Strukturen untersucht. In Deutschland ist neben der künstlichen Bestandsbegründung die natürliche Verjüngung eine überwiegend waldbaulich praktizierte Form der Waldregeneration, die es zulässt das genetische Erbgut zu erhalten. Neben zur Selbstdifferenzierung quantitativ ausreichend und gut verteilter Individuen in der Verjüngungsschicht sind für die Qualität und Vitalität des Bestandes die genetische Variation und räumlich genetische Strukturen relevant. Für die dritte Studie wurden daher zwei slawonische und zwei einheimische Bestände sowie deren Naturverjüngung sowohl an 23 Kern-DNA-Markern als auch an 5 Chloroplasten-DNA-Markern genetisch charakterisiert. Die Ergebnisse zeigen, dass die genetische Information von Altbeständen fast vollständig an die nächste Generation weitergegeben wird. Der Grund hierfür liegt in dem ähnlich hohen Maß an genetischer Variation, einer geringen genetischen Differenzierung sowie einer ähnlichen bis übereinstimmenden Haplotypen-Zusammensetzung wie sie die jeweiligen Altbestände aufzeigen. Da sich die genetische Vielfalt stark auf die Anpassungsfähigkeit von Populationen an sich ändernde Umweltbedingungen und ihr Überleben auswirkt, kann durch eine hohe genetische Variabilität eine Stabilität und Nachhaltigkeit von Waldökosystemen gewährleistet werden. Darüber hinaus gab es keine Hinweise auf Hybridisierung oder Genfluss zwischen slawonischen und einheimischen Populationen. Ein naheliegender möglicher Grund hierfür ist der unterschiedliche Austriebzeitpunkt der untersuchten Taxa.

Das letzte Kapitel der Dissertation (Kapitel 5) befasst sich mit der Erstellung der ersten auf RADseq (restriction site-associated DNA sequencing) - SNP-Markern basierenden, hochauflösenden, genetischen Kopplungskarte (männlich und weiblich) innerhalb der intraspezifischen *Quercus robur* - Vollgeschwisterfamilie (*Q. robur* x *Q. robur* ssp. *slavonica*). Zur Erstellung der weiblichen und männlichen genetischen Kopplungskarten wurden 249 Nachkommen der kontrollierten intraspezifischen Kreuzung *Q. robur* x *Q. robur* ssp. *slavonica* verwendet. Dabei wurden die genetischen

Kopplungskarten unter der Anwendung der *Pseudo-Testcross*-Methode und mit Hilfe *Kosambis* Kartierfunktion erstellt. Insgesamt wurden 473 SNP-Marker auf der weiblichen und 502 SNP-Marker auf der männlichen Kopplungskarte kartiert, die jeweils aus 12 Kopplungsgruppen bestehen. Diese Karten bilden u. a. eine wertvolle Grundlage für die Durchführung zukünftiger QTL (Quantitative Trait Loci) - Analysen, Identifizierung von an der Variation adaptiver Merkmale beteiligter Gene sowie für vergleichende Genomanalysen.

# Einleitung

# 1. Einleitung

Seit Jahrzehnten wachsen Bewusstsein und Verständnis für einen nachhaltigen Umgang und dauerhaften Erhalt der Natur in unserer Gesellschaft und führen damit immer häufiger zu öffentlichen und politischen Diskussionen. Der Klimawandel mit seiner derzeit rasch ablaufenden Geschwindigkeit sowie einhergehenden Auswirkungen entwickeln sich aktuell zu den größten Stressfaktoren von Waldökosystemen. Die Dokumentation des Klimawandels ist international bis in die Zeit der Industrialisierung (um 1850) gut dokumentiert, sodass sich eine Erwärmung der Erdoberfläche um durchschnittlich rund 1 °C (2006-2015) nachweisen lässt (IPCC 2018). Bis zum Jahr 2100 ist laut IPCC (2014) ein mittlerer globaler Temperaturanstieg zwischen 1,6 und 4,6 °C zu erwarten. Die Erwärmung ist maßgeblich auf eine Treibhausgaserhöhung in der Atmosphäre und diese wiederum auf anthropogene Einflüsse, wie die zunehmende Verbrennung fossiler Brennstoffe, die umfangreiche Nutztierhaltung, einen zunehmenden Einsatz von Düngemitteln und die Abholzung von Wäldern (Roshani et al. 2022), zurückzuführen. Mit einem weltweiten industriellen Wachstum werden immer neue Ansätze zur Verringerung des Ausstoßes und zur Speicherung der Kohlenstoffdioxid-Emissionen benötigt, um voranschreitende klimatische Veränderungen zu verlangsamen. Wälder zählen nach den Ozeanen, zu den natürlichen Hauptsenken des Treibhausgases Kohlenstoffdioxid (Fischlin et al. 2006) und tragen über die Ökosystemdienstleistung der Kohlenstoffbindung erheblich zum Klimaschutz bei.

Deutschland ist nach Zahlen der letzten Bundeswaldinventur auf 11,4 Mio. ha und damit zu knapp einem Drittel der Fläche bewaldet (BMEL, 2014). Es zählt zu den waldreichsten Ländern Europas, wobei ca. 56,4 % der bewaldeten Fläche auf Nadel- und 43,6 % auf Laubwald entfallen (Kändler, 2015). Allein die Waldbewirtschaftung und die Verwendung des bereitgestellten Holzes verringern die deutsche Treibhausgasbilanz aktuell um ca. 11 - 14 % (Schulze et al. 2021). In Abhängigkeit der Baumartenzusammensetzung besitzen Wälder weitere unterschiedlich stark ausgeprägte Nutz-, Schutz- und Erholungsfunktionen. Sie produzieren den wichtigsten nachwachsenden Rohstoff Holz (Noack und Köhn 2021), beeinflussen Wasserregulierung und Erosionsschutz (Abildtrup et al. 2013; Thompson et al. 2011), beherbergen biologische Vielfalt (Mori et al. 2017) und dienen der menschlichen Erholung (Hegetschweiler et al. 2007). Dass der Wald neben den standörtlichen Bedingungen und der Bewirtschaftung im Wesentlichen von den klimatischen Faktoren, vor allem Niederschlag und Temperatur (Lorenz et al. 2008, LWF 2019, Nicolussi 2006) beeinflusst wird, erschwert die aktuelle Bewirtschaftung und einen langfristigen Erhalt. So bedingt die globale Erderwärmung bereits jetzt die Zunahme von Dürren (u. a. Dürresommer 2018) sowie Stürmen und führt zu einer Mehrung von Vegetations- und Waldbränden (Allen et al. 2015, Andela et al. 2017, Schuldt et al. 2020). Die durch Witterungsextreme fortlaufend geschwächten Bestände sind zudem anfälliger für Schadinsekten und Krankheitserreger (Weed et al. 2013). Zwar sind Waldökosysteme

schon seit je her durch Störungen geprägt, die häufig auch zur Artenvielfalt und heterogeneren Landschaften beitragen (Buma and Schultz 2020). Sowohl jedoch die Geschwindigkeit mit der der Klimawandel aktuell abläuft als auch die Auswirkungen, die bereits im Jahr 2022 ein historisches Ausmaß erreicht haben, bringen Zweifel auf, ob sich die Waldökosysteme genügend rasch an die veränderten Umweltbedingungen anpassen und ihre Ökosystemleistungen dauerhaft erfüllen können (Anderegg et al. 2020, Gauthier et al. 2015, Pluess et al. 2016).

Neben dem Walderhalt ist daher ein Erhalt der Anpassungsfähigkeit von Baumpopulationen im Waldökosystem elementar. Aufgrund ihrer Langlebigkeit und Immobilität sind Bäume im Laufe ihres Lebens zwangsläufig verschiedenen zeitlich veränderlichen und räumlich heterogenen Umweltbedingungen ausgesetzt, die sie überleben und bewältigen müssen (Vornam et al. 2004). Dabei sind Anpassungen an diese stark veränderlichen lokalen Umweltbedingungen zwingend notwendig. Der Phänotyp eines Baumes ergibt sich aus dem komplexen Zusammenwirken der Erbanlagen (Genotyp) und der Umwelt. Somit stehen Waldbäume im ständigen Austausch mit ihrer Umgebung. Die genetische Ausstattung und die Umweltfaktoren des Wuchsortes bestimmen hierbei die Angepasstheit des Baumes (Finkeldey and Hattemer, 2010). Die gegenwärtig herrschenden und wechselnden abiotischen (z.B. Licht, Temperaturverhältnisse, Boden-pH-Wert, Wasserverfügbarkeit) und biotischen (z.B. intra- & interspezifische Konkurrenz) Einflüsse führen zu unterschiedlichem Wachstum innerhalb und zwischen Baumarten, wobei diejenigen Individuen überleben, die besser angepasst sind (Bartsch und Röhrig, 2016). Dabei wird die Anpassung an standörtliche Begebenheiten durch diverse Prozesse, wie phänotypische Plastizität, Gendrift und natürliche Selektion, bestimmt. Eine Grundlage für fortlaufende Anpassungsprozesse stellt die genetische Variation dar (González-Martínez et al. 2006, Krutovsky und Neale 2005). Populationen mit geringer genetischer Vielfalt sind anfälliger für das Auftreten neuer Schädlinge oder Krankheitserreger und damit auch gegenüber veränderlicher Umweltbedingungen. Populationen mit einer breiten genetischen Variation hingegen können besser auf die aktuellen und prognostizierten, extremen Klimabedingungen reagieren. Deshalb sind sowohl das Vorhandensein als auch das Fortbestehen der genetischen Variation wichtig für den langfristigen Erhalt, die Stabilität und die Leistungsfähigkeit der Waldbestände (Vornam et al. 2004).

## 1.1 Molekulare Marker

Die Prüfung der lokalen Anpassung von Populationen und die Vorhersage ihrer Reaktion auf die künftige Umwelt sind im Hinblick auf den Klimawandel von zentraler Bedeutung (Rellstab et al. 2016). Um die genetische Vielfalt und Anpassung bei Waldbäumen untersuchen zu können, werden Provenienzversuche und molekulargenetische Marker verwendet (González-Martínez et al. 2006). Herkunftsversuche dienen dazu, langfristig Informationen über das Anpassungspotential

verschiedener Baumarten und Provenienzen an das künftige Klima zu erhalten (Fotelli, 2021) und gleichzeitig die Anbaueignung der jeweiligen Baumart auf verschiedenen Standorten zu prüfen.

Molekulare Marker, auch DNA-Marker genannt, sind kurze eindeutig identifizierbare DNA-Abschnitte, die Variationen zwischen Individuen aufweisen und mit denen Polymorphismen für eine bestimmte DNA-Frequenz innerhalb einer Population nachgewiesen werden können (Jiang, 2013). Zudem ist ihre Lage im Genom bekannt. Es können verschiedene Markertypen verwendet werden, die sich je nach Nachweisverfahren (basierend auf Hybridisierung oder PCR), Art der Übertragung (mütterliche, väterliche oder biparentale Vererbung), Durchsatz (gering oder hoch) und Art der Wirkungsweise des Gens (kodominant oder dominant) klassifizieren lassen (Mammadov et al. 2012; Nadeem et al. 2018). Einige Beispiele für DNA-Marker sind Restriktionsfragmentlängen-Polymorphismus (**RFLPs**, engl.: restriction fragment length polymorphisms), zufällig amplifizierte polymorphe DNA (**RAPD**, engl.: Random Amplification of Polymorphic DNA), amplifizierter Fragmentlängen-Polymorphismus (**AFLPs**, engl.: Amplified Fragment Length Polymorphism), einfache Sequenzwiederholungen (**SSRs**, engl.: Simple Sequence Repeats) und Einzelnukleotid-Polymorphismus (**SNPs**, engl.: Single Nucleotide Polymorphism). Unter diesen genannten DNA-Markern werden die SSR-Marker in populationsgenetischen Analysen bevorzugt verwendet, da sie in der Regel ko-dominant sind, einen hohen Polymorphismusgrad besitzen, gleichmäßig über das gesamte Genom verteilt und reproduzierbar sowie artenübergreifend übertragbar sind (Durand et al. 2010, Ellis and Burke 2007, Guichoux et al. 2011, Li et al. 2022). SSR-Marker können als genetische (EST-SSRs) oder genomische (gSSRs) Marker klassifiziert werden (Vašek et al. 2020).

Gleichzeitig entwickelt sich in jüngerer Vergangenheit mit dem Einsatz von Einzelnukleotid-Polymorphismen (SNPs), eine brauchbare Alternative zur Genotypisierung von Baumarten.
SNP-Marker stellen Unterschiede (Variationen) in den einzelnen Nukleotiden an einer bestimmten Sequenzposition zwischen Individuen in einer Population dar (Wang et al. 1998). Ähnlich wie die SSR-Marker sind SNPs kodominant, hoch polymorph und kommen im gesamten Genom (ca. alle 500-1000 bp) vor (Chen et al. 2019). Mit der Einführung (seit 2004) von Hochdurchsatz-Sequenzierungstechnologien der nächsten Generation (NGS=next generation sequencing) und seither stetigen Verbesserungen hin zu schneller, effizienter und kostengünstiger DNA-Sequenzierung gewann die Genotypisierung durch Sequenzierung (GBS=genotyping by sequencing) in den letzten Jahren zunehmend an Popularität (Barba et al. 2014). Die verschiedenen NGS-Technologien unterscheiden sich darin, wie die Reads erfasst, amplifiziert und sequenziert werden (Quail et al. 2012). Dabei sind die gesamte Genomsequenzierung sowie die Ansätze zur Reduzierung des Genomkomplexes, wie RADseq (Restriktionsstellen-assoziierte DNA Sequenzierung), die beiden Hauptmethoden der Sequenzierung (Baird et al. 2008). Die Entwicklung von RADseq ermöglichte in

den letzten Jahren auch die Untersuchung genomweiter genetischer Muster in Nicht-Modellbaumarten (Parchman et al. 2018). Dabei kann RADseq gleichzeitig Tausende von genetischen SNP - Markern identifizieren und bewerten, die zufällig über das Zielgenom verteilt sind (Davey und Blaxter 2010).

## 1.2 Die Gattung *Quercus*

Eichen (*Quercus* L., Fagaceae) zählen zu den ökonomisch und ökologisch wichtigsten Baumarten in Europa. Die Gattung *Quercus* bildet dabei mit etwa 500-530 anerkannten Eichenarten die artenreichste Gattung innerhalb der Buchengewächse (Fagaceae) (Jablonski, 2015), wobei die Stiel- (*Quercus robur* L.) und die Traubeneiche (*Quercus petraea* (Matt.) Liebl.) in Europa am weitesten verbreitet sind. Ihr Vorkommen erstreckt sich von Nordspanien bis in den Süden Skandinaviens und von Irland bis nach Osteuropa. Sie wachsen unter einer Vielzahl von klimatischen Bedingungen (ozeanisch bis kontinental), in verschiedenen Höhenlagen bis auf 1800 m ü. M. sowie auf unterschiedlichen, von feucht bis trocken und alkalisch bis sauer reichenden Standorten (Ducousso and Bordacs 2004, Günthardt-Goerg et al. 2016, Kesić et al. 2021, Kremer 2010). Diese Vielfalt an Lebensräumen (von Auenwäldern bis Graslandschaften, (Abrams 1992)), spiegelt das wertvolle genetische Potential der Eichen wider (Günthardt-Goerg et al. 2016, Kremer 2010). Darüber hinaus zählen Eichen zu den Baumarten in Europa, die überdurchschnittlich variabel sind (Bonfils et al. 2015). Sie weisen eine relativ hohe genetische Variation innerhalb ihrer Populationen und eine relativ geringe Variation zwischen den Populationen auf (z.B. Burger et al. 2021, Burger and Gailing 2021, Kesić et al. 2021, Spence et al. 2021). Mögliche Gründe hierfür ergeben sich aus dem großen geografischen Verbreitungsgebiet der Eichen, ihrem auskreuzenden Zuchtsystem, ihrer Langlebigkeit sowie sowohl der wind- als auch tiergestützten (z.B. durch Eichelhäher) Samenverbreitung (Hamrick et al. 1992). Diese hohe genetische Variation innerhalb von Eichenpopulationen bildet die wichtigste Voraussetzung, sich an komplexe und stetig ändernde Umweltverhältnisse anzupassen, um langfristig überleben und die genetische Variation an die nächste Generation weitergeben zu können. Daher sind Eichen seit langem ausgezeichnete Modellbaumarten in der Evolutionsgenetik, um die Anpassung von Waldbäumen an veränderte Umweltbedingungen und derzeit insbesondere im Hinblick auf den Klimawandel zu untersuchen.

## 1.3 Forschungsvorhaben und Zielsetzung

Mit voranschreitender Klimaerwärmung reichen bisherige Anbauempfehlungen für einen Waldumbau hin zu klimastabilen Wäldern allein möglicherweise nicht aus. Kenntnisse sowohl über die genetische Vielfalt als auch die Anpassungsfähigkeit von Populationen bzw. Herkünften sind wichtiger als je zu vor, um Jahrhunderte dauernde Anpassungsprozesse zu beschleunigen. Mit der slawonischen

Stieleiche (*Quercus robur* subsp. slavonica (Gáyer) Mátyás (siehe Kapitel 2)) haben wir eine für deutsche Wälder interessante Art mit hohem forstlichen Potential. Derzeit behindern u. a. fehlende Erfahrungen sowie die geringe Anzahl an Studien zur genetischen Charakterisierung einen zunehmenden Anbau. Ziel dieser Arbeit ist es daher, einen Teil zur genetischen Charakterisierung und langfristig zur forstlichen Akzeptanz dieser Stieleichenvarietät beizutragen.

In Kapitel 2 wird die Eignung der slawonischen Stieleiche als Varietät aufgrund ihrer Eigenschaften und ihres Potentials als Alternative für einen klimastabilen Waldumbau dargestellt. Das Ziel in Kapitel 3 ist es, die vorhandenen slawonischen Stieleichenbestände in Westdeutschland von einheimischen Stieleichenpopulationen mithilfe von Kernmarkern genetisch zu differenzieren. Diese sollen eine potentielle Verringerung der genetischen Variation durch die Samenübertragung der slawonischen Stieleiche durch den Menschen nach Deutschland aufdecken. In Kapitel 4 werden benachbarte slawonische und einheimische Bestände auf Genfluss, Hybridisierung und den Erhalt der genetischen Variation in der Naturverjüngung untersucht. Diese Kenntnis ist wichtig, da ein Erhalt der genetischen Vielfalt die Wahrscheinlichkeit erhöht, gut angepasste Genotypen zu haben, die eine langfristige Lebensfähigkeit von Waldbäumen gewährleisten. Für das letzte Kapitel dieser Arbeit (Kapitel 5) werden genetische Kopplungskarten basierend auf RADseq-Markern erstellt. Diese dienen als Grundlage für zukünftige Studien, um Genomregionen identifizieren zu können, die mit phänotypischen Eigenschaften (z.B. Blattaustrieb, Trockentoleranz) im Zusammenhang stehen.

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Kapitel 2

# Die slawonische Stieleiche (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) als Alternative für den Klimawandel: Experimentelle und genomische Ressource

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# Die slawonische Stieleiche (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) als Alternative für den Klimawandel: Experimentelle und genomische Ressource

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**Beiträge der Autoren** Katrin Burger hat wesentlich zum Konzept und Design der Studie beigetragen und war für die Literaturrecherche sowie das Verfassen des Manuskriptes verantwortlich. Sie erstellte auch die Tabelle. Oliver Gailing überarbeitete das Manuskript kritisch auf wichtige intellektuelle Inhalte. Alle Autoren haben das Manuskript durchgesehen und die eingereichte Version genehmigt.

## Zusammenfassung

Die slawonische Stieleiche wurde erst Ende des 19. Jahrhunderts mit Beginn des Samentransportes über weite Strecken im Zuge des Schienennetzausbaus nach Deutschland eingeführt. Der Ursprung des genetischen Materials liegt vermutlich in den Save-Niederungsgebieten im Osten des heutigen Kroatiens. Sie zeichnet sich im Vergleich zur einheimischen Stieleiche besonders durch ihren späten Blattaustrieb sowie ihre Geradschaftigkeit und stärkere Wuchsleistung aus. Obwohl die slawonische Stieleiche aufgrund ihrer aus ertragskundlicher Sicht sehr guten Eigenschaften in der Forstwirtschaft immer mehr an Bedeutung gewinnt, befassten sich in jüngster Vergangenheit erst wenige Studien mit der genetischen Variation und/oder Merkmalsvariation dieser Art. Das Ziel dieses Übersichtsartikels ist eine umfassende Betrachtung der slawonischen Stieleichen als alternative Baumart für den Waldumbau im Hinblick auf die potenzielle Anpassungsfähigkeit an den prognostizierten Klimawandel. Die aktuell für die Forstwirtschaft relevantesten Erkenntnisse zur slawonischen Stieleiche sollen zusammenfassend dargestellt werden, um eine Grundlage für zukünftigen Forschungsarbeiten zu bieten. Im Rahmen eines Herkunftsversuches aus 22 österreichischen und kroatischen Stieleichenpopulationen ließen sich signifikante Unterschiede in der Überlebensrate sowie Höhenwachstum als Zeichen lokaler Anpassung an den Wasserhaushalt ableiten. Die höchsten Überlebensraten wiesen Populationen aus trockenen Lebensräumen Kroatiens auf.

Zudem konnten aus Untersuchungen zu genetischer Variation sowie räumlich genetischer Strukturen bei slawonischen Stieleichen zeitlich unterschiedliche Knospenaustriebe zwischen den Stieleichen-Varietäten festgestellt und eine Korrelation zwischen dem Austriebszeitpunkt und der genetischen Variation im Chloroplastengenom nachgewiesen werden. Anhand der räumlichen Variation genetischer Strukturen der Stieleiche konnten mithilfe molekulargenetischer Marker Herkünfte der slawonischen Stieleiche eindeutig identifiziert werden. Zudem können QTL-Kartierungen genutzt werden, um die genetischen Grundlagen der Differenzierung zwischen den Taxa (*Q. robur* subsp. *robur* und *Q. robur* subsp. *slavonica*) zu klären. In zukünftigen Untersuchungen werden wir mit Hilfe der genomweiten genetischen Kartierung und Assoziationsanalysen in Herkunftsversuchen die genetische Basis der Wuchsleistung und Stammqualität der slawonischen Stieleiche in Deutschland sowie die mögliche Übertragung wünschenswerter Merkmale durch die Hybridisierung mit der einheimischen Stieleiche untersuchen.

## Summary

**Slavonian pedunculate oak (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) as an alternative for climate change: experimental and genomic resources**

The Slavonian oak was introduced into Germany at the end of the 19th century with the beginning of seed transport by the expansion of railway connections. The origin of the genetic material probably lies in the Sava lowlands in the east of today's Croatia. Compared to the common oak, it is characterized especially by its late bud burst as well as its straightness and stronger growth performance. Slavonian pedunculate oak is becoming increasingly important in forestry due to its very good characteristics from a yield point of view. However, only a few studies were conducted on the genetic variation and/or trait variation of this species. The objective of this review is to give an overview on Slavonian oak as an alternative variety for forest restructuring in terms of its adaptability to predicted climate change. The findings on Slavonian oak that are currently most relevant to forestry are summarized to provide a basis for future research. Based on studies of height growth and survival of 22 Austrian and Croatian pedunculate oak populations in a provenance trial, significant differences in survival as well as height growth could be determined and local adaptations to the water regime could be deduced. Populations from dry regions in Croatia showed the highest survival rates.

In addition, studies on genetic variation as well as spatial genetic structures in Slavonian oaks showed differences in timing of vegetative bud burst between pedunculate oak varieties, and based on this, statistical associations between bud burst and genetic variation in the haploid plastids, as an indicator of the geographic origin in Croatia, were detected. Nuclear microsatellites were used to differentiate between native pedunculate and Slavonian oak stands. In addition, QTL mapping can be used to clarify the genetic basis of differentiation between taxa (*Q. robur* subsp. *robur* und *Q. robur* subsp. *slavonica*).

In future studies, we will use genome-wide genetic mapping and association analyses in provenance trials to investigate the genetic basis of growth performance and stem quality of Slavonian oak in Germany, as well as possible transfer of desirable traits through hybridization with native oak.



**Schlagwörter – Key words**

Slawonische Stieleiche, genetische Variation, Sonderherkunft, QTL, Assoziationskartierung

Slavonian oak, genetic variation, special origin, QTL, association mapping

## 2.1 Einleitung

Eichen (Gattung *Quercus*, Familie der Fagaceae) gehören zu den häufigsten und weitverbreitetsten Baumarten und stellen eine ökologisch sowie ökonomisch wichtige Ressource in den subtropischen, mediterranen und gemäßigten Gebieten der nördlichen Hemisphäre dar (Dumolin et al. 1995, Barreneche et al. 1998, Curtu et al. 2007, Aldrich und Cavender-Bares 2011). In Deutschland sind etwa 10,3 % der Waldfläche (1.129.706 ha) mit Eiche bestockt. Damit bildet sie die 4. häufigste Baumartengruppe nach Fichte, Kiefer und Buche (Bundesministerium für Ernährung und Landwirtschaft (BMEL) 2016). Innerhalb der Gattung *Quercus* dominiert die Stieleiche (*Quercus robur* L.) in Deutschland und nahezu in ganz Europa und gehört damit zu den wichtigsten Laubbaumarten (Barreneche et al. 1998, Reif et al. 2016). Ihr Verbreitungsgebiet erstreckt sich im Süden vom nördlichen Teil Spaniens bis in den Süden Skandinaviens und im Westen von Irland bis ins Ural-Gebirge Russlands (Eaton et al. 2016, EUFORGEN 2020). Sie besitzt eine breite ökologische Standortsamplitude, die von nassen bis trockenen sowie von sauren bis hin zu alkalischen Standorten reicht (Tschermak 1950, Ellenberg 1963, Raus 1986, Riek 2006, EUFORGEN 2020). Die Stieleiche wächst vorrangig auf nassen bis feuchten, nährstoffreichen Böden sowie in niedrigeren bis mittleren Höhenlagen (Eaton et al. 2016). In Augebieten (nährstoffreiche, tiefgründige, gut mit Wasser versorgte Lehm- und Tonböden) erreicht sie ihr Optimum und verträgt sogar periodische Überschwemmungen (Raus 1986, Riek 2006). Insgesamt tragen Eichen zum Erhalt der Biodiversität durch eine Erhöhung der Artenvielfalt in den Wäldern bei, da sie wie kaum eine andere Baumart von zahlreichen, häufig geschützten, Insekten- und Pilzarten besiedelt werden (Blaschke und Nannig 2014, Bußler 2014). Die Stieleiche gehört jedoch zu den Arten, die besonders durch menschliches Einwirken, wie beispielsweise Samentransport, künstliche Verjüngung, selektiver Holzeinschlag oder Verfrachtung betroffen ist (Bordács et al. 2002). Innerhalb der Art *Quercus robur* haben sich mit der Zeit zahlreiche taxonomische Unterarten bzw. ökologische Varietäten in geographischen Teilgebieten des gesamten Verbreitungsgebiets herausgebildet, wie z. B. *Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky mit Verbreitungsgebiet von Griechenland bis Rumänien sowie Kleinasien (Bordács et al. 2002, Bussotti und Grossoni 2008) oder *Quercus robur* subsp. *slavonica* (Gáyer) Mátyás mit Verbreitungsgebiet in Slawonien (Kroatien). Letztere ist bis heute vor allem aufgrund ihrer sehr guten Qualitäten in ganz Europa als Importholz begehrt (Rieger 2018) und erlangt aktuell durch den Klimawandel bedingten Waldumbau vermehrt als Saatgut an Interesse für die Forstwirtschaft. Das Ziel dieser Arbeit ist daher, die slawonische Stieleiche (*Quercus robur* subsp. *slavonica*) als Alternative für den Waldumbau im Hinblick auf den Klimawandel darzustellen. Hierfür werden im Folgenden die relevantesten Ergebnisse in Bezug auf forstgenetische Ressourcen (Anpflanzungen, Identifikation von Herkünften, QTL- und Assoziationsanalysen) und genomische Ressourcen (Genomsequenz, Transkriptome, genetische Marker, Kopplungskarten und Kandidatengene) der slawonischen Stieleiche aufgezeigt.

### 2.1.1 Slawonische Stieleiche - Allgemeines

Die slawonische Stieleiche wurde von ungarischen Botanikern identifiziert und taxonomisch als Unterart (*Quercus robur* subsp. *slavonica)* eingestuft (Mátyás 1970). Zu dieser Zeit mangelte es jedoch an gründlichen Untersuchungen der Baumarten im Hinblick auf häufige Hybridisierung, ökologische Ansprüche, natürliche vs. künstliche Areale und Zwischenformen (Bartha 2010). Dies lag im Fall der slawonischen Stieleiche zum einen daran, dass durch den exportbedingten Holzeinschlag viele Flächen mit verschiedenen Waldbildern zerstört und mit anderen Arten verjüngt wurden (Rieger 2018) und zum anderen an mangelnden Forschungsmethoden und Ansätzen, die Arten genetisch ausreichend zu untersuchen. Mit fortschreitender Forschung ergaben sich hieraus zunehmende Unklarheiten in der Zuordnung von Unterarten, sodass heute auch die Klassifizierung der slawonischen Stieleiche als Unterart in Frage gestellt werden muss. Autoren wie Bartha (2010) charakterisierten in einschlägiger Literatur die slawonische Stieleiche daher als Form, Varietät oder Ökotyp der Stieleiche. Solche Fehler bei der Identifizierung von Arten und ihrer taxonomischen Einordnung sind, sofern sie sich erst einmal durchgesetzt haben, nur schwer zu korrigieren (Bartha 2010). So wird bis heute in der Fachliteratur die Bezeichnung *Quercus robur* subsp. *slavonica* als Synonym für die fachlich korrekte Bezeichnung *Quercus robur* var. *slavonica* verwendet.

Der Ursprung der slawonischen Stieleiche ist auf die Niederungen der Flüsse Save, Drau, Bosut und Donau zwischen Zagreb und Belgrad im Osten Kroatiens (Region Slawonien) zurückzuführen (Gailing et al. 2007a, b, 2003, Gehle 1999, Hesmer 1955, Wachter 2001, 2011). Auch, weil in dieser Region vor allem ihr großer Bedarf an Grundwasser dauerhaft gedeckt werden konnte (Rieger 2018). Denn ihr standörtliches Optimum sind tiefgründige, lehmige sowie sandige und durch Wasser beeinflusste, fruchtbare Böden (Raus 1986).

Bis ins Zeitalter der Industrialisierung war die Fläche Ostslawoniens zu 75 % mit Stieleichenwäldern bestockt. Aktuell sind es nur noch knapp 35 % der Fläche (Rieger 2018). Gründe hierfür ergeben sich neben der starken Übernutzung der Wälder vor allem aus der Wassernutzung des Menschen für die Landwirtschaft, mit einem hierdurch bedingten kontinuierlichen Abfall des Grundwasserspiegels. Heute erstreckt sich ihr natürliches Verbreitungsgebiet über mehr als 400 km Länge in Höhenlagen zwischen 80 und 200 m ü. NN entlang der Save-Niederung (16- 20 ° Längengrad, 45-46 ° Breitengrad) mit daraus resultierenden erheblichen klimatischen Unterschieden (Wachter 2011). Insgesamt wird das Klima in der Save-Niederung als ein vom Mittelmeer beeinflusstes gemäßigtes kontinentales Klima beschrieben, in dem die Vegetationsperiode aufgrund der kurz verlaufenden Jahreszeiten Frühling und Herbst länger ausfällt und die Sommermonate von häufigen oft starken Regenfällen geprägt sind. Zwischen Sommer- und Wintermonaten herrschen zudem stark unterschiedliche Temperaturen (Fischer 2005). Durchschnittlich beträgt die Jahrestemperatur 10-

12 °C, die jährlichen Niederschläge schwanken in Ostslawonien zwischen 500 und 800 mm, in Westslawonien fallen 900-1000 mm Niederschlag, wobei Regenfälle insgesamt vor allem während der Vegetationsperiode auftreten (Fischer 2005, Mühr 2021, Raus 1986, Steiner 1957). Die natürlichen Waldgesellschaften, in denen die slawonische Stieleiche in Kroatien heute vorkommt, sind die Hartholzaue, der seggenreiche Stieleichen-Auwald und der Stieleichen-Hainbuchenwald (Glavac 1969, Wachter 2011). Die vorherrschenden Standorte der Hartholzaue und des seggenreichen Stieleichen-Auwalds werden auch als Extremstandorte beschrieben, auf denen sich nur vereinzelte, angepasste Arten etablieren können. Außerhalb der Aue wächst sie heute nur auf Standorten, auf denen die Konkurrenzkraft der Buche herabgesetzt ist (Glavac 1969). Innerhalb der Waldgesellschaft Stieleichen-Hainbuchenwald hat sich ein Großteil der Bestände durch herauswachsen der Hutewälder gebildet.

In Deutschland wurde die slawonische Stieleiche mit der Etablierung der Dampfmaschinen im 19. Jahrhundert eingeführt. Erst in Form von Rundholz sowie Sägeprodukten (Rieger 2018) und später zum Ende des 19. Jahrhunderts mit Beginn eines extensiven Saatguthandels als Saat- und Pflanzgut (Gailing et al. 2007a, b, Wachter 2001). Besonders in der Region um Münster (so genanntes „Münsterland“) wurden zu dieser Zeit einige Bestände (ca. 87,6 ha) mit slawonischem Saatgut künstlich begründet (Wachter 2001, 2011). Dies geschah zwischen 1870-1900 nicht aus Überzeugung, sondern viel mehr aus der Not heraus. So war die deutsche Forstwirtschaft in vorher nicht bekanntem Maße vom Eichensterben sowie schlechten Mastjahren geplagt, sodass vor allem größere Forstbetriebe für ihre Planungssicherheit, und um nicht auf den Eichenanbau verzichten zu müssen, auf importierte Eicheln setzten (Mortzfeldt 1896). Diese Flächen im Niederungsgebiet Westfalens und des Niederrheins (6-8° Längengrad, 51-52° Breitengrad) befinden sich ca. 1000 km nordwestlich des Ursprungsgebiets und sind durch ein ozeanisch geprägtes Klima mit milden Wintern, geringen Temperaturschwankungen, einer relativ langen Vegetationsperiode (>240 Tagen) und einem Jahresniederschlag zwischen 700 und 800 mm charakterisiert (Klimaatlas NRW 2021, WALDINFO NRW 2021, Wachter 2011). Die natürlichen Waldgesellschaften, in denen die slawonische Stieleiche heute in Deutschland vorkommt, sind der Flattergras-Buchenwald, der Bucheneichenwald und der Eichenbirkenwald (Wachter 2011). Im direkten Vergleich zu heimischen Herkünften sind die slawonischen Stieleichen aus Nordrhein-Westfalen heute gut waldwachstumskundlich sowie genetisch untersucht und übertreffen auf ausreichend wasserverfügbaren Standorten heimische Stieleichen sowohl im Wachstum als auch in der Qualität sowie hinsichtlich der insektenbedingten Krankheitsunanfälligkeit (siehe Kapitel 1.2). Aus Erfahrungen des Landesbetriebes Wald und Holz NRW trifft dies gleichermaßen für Standorte im Tiefland und Mittelgebirge zu (Wald und Holz NRW 2014a). Weiterhin existieren in Deutschland in den Bundesländern Hessen, Niedersachsen und Sachsen-Anhalt kleinere Bestände mit slawonischen Stieleichen (insgesamt ca. 17,5 ha), die allerdings bisher noch nicht genetisch untersucht wurden. Die forstliche Eignung der slawonischen Stieleiche auf verschiedenen

Bodensubstraten von 25-260 m ü. NN in den Wuchsgebieten Nordrhein-Westfalens konnte über den Zeitraum der letzten 100 Jahre gezeigt werden (Wald und Holz NRW 2014a). Damit verfügt sie nachweislich über eine wesentlich breitere Standortsamplitude als dies bisher aus ihrem Ursprungsgebiet bekannt war.

### 2.1.2 Merkmale

Besondere forstliche Aufmerksamkeit erlangte die slawonische Stieleiche in Deutschland erst aufgrund ihres hier festgestellten späteren Blattaustriebs. Untersuchungen zum Austriebszeitpunkt wurden von Wachter (2001) und Gailing et al. (2003) in verschiedenen nordrhein-westfälischen Beständen durchgeführt und zeigten bei slawonischen Provenienzen einen bis zu 3 Wochen späteren Austrieb verglichen mit den autochthonen einheimischen Stieleichen. Die Bestände der slawonischen Stieleiche wurden hierdurch häufig als „Späteichen" beschrieben. Heute ist bekannt, dass auch innerhalb der slawonischen Stieleichen eine Zeitspanne im Austrieb (früh bis spät) besteht, was sich allerdings bisher nicht negativ auf den besseren Wuchs auswirkt (Wald und Holz NRW 2014a).

Der spätere Austrieb wird in Deutschland als natürlicher Schutz gegen Spätfrost sowie gegen Schädigungen durch den Eichenwickler angesehen (Wachter 2001, Gailing et al. 2003). Positiv könnten sich zudem die kühleren Temperaturen im künstlichen Anbaugebiet auswirken, da stark wärmeabhängige Schadinsekten, wie beispielsweise der Schwammspinner (*Lymantria dispar,* Hauptschädiger an der Stieleiche in Slawonien) in Deutschland bisher nicht schädigend auftreten (Wachter 2011). Erfahrungen aus den Beständen Nordrhein-Westfalens bestätigen dies und zeigen zudem, dass die slawonischen Stieleichen bisher kaum von der Eichenfraßgesellschaft sowie dem häufig nachfolgenden Mehltaublattpilz befallen werden (Wald und Holz NRW 2014b).

Neben dem späteren Blattaustrieb lässt sich die slawonische Stieleiche vor allem anhand ihres besonderen Erscheinungsbildes von anderen Eichen unterscheiden (Wachter 2011). Zu ihren phänotypischen Besonderheiten zählen Brettwurzeln, welche vor allem der Stabilisierung auf Standorten mit hoher Grundwasserbeeinflussung dienen, eine relativ glatte, gleichmäßige Borke, das Fehlen von Frostrissen und sehr selten vorkommende Wasserreißer.

Aus ertragskundlicher Sicht zeichnet sie sich vor allem durch ihre sehr hohe Wuchsleistung, Geradschaftig-, Vollholzig-, Steilastig- und Schmalkronigkeit aus. Das Holz der slawonischen Stieleiche ist aufgrund der guten Bearbeitungsfähigkeit und Beschaffenheit, der großen Stammholzdimensionen, der gleichmäßigen Jahrringe und der Holzkonzentrizität vor allem als Schnitt- und Furnierholz sehr beliebt (Wachter 2011). Im Hinblick auf die holzanatomischen und - physiologischen Merkmale Dichte, Kernholzanteil, Quell- und Schwindverhalten übertrifft die slawonische Stieleiche die heimische Stieleiche teils stark (Peters und Becker 1995). Untersuchungen von Wachter (2011) aus dem Jahr 2008

ergaben eine Überlegenheit der slawonischen Stieleiche (110-130-jährig) in Höhe, Grundfläche und Durchmesser. Höhere Massenzuwächse und Qualitätsleistungen im direkten Vergleich der beiden Eichentaxa in Reinbeständen, aber auch bei unterschiedlicher waldbaulicher Behandlung am Beispiel Untersuchungsgebiet Kottenforst, bestätigt auch der Landesbetrieb Wald und Holz NRW (Wald und Holz NRW 2014a). Aufgrund ihrer vielen, den Nachteilen überwiegenden Vorteile gilt die slawonische Stieleiche in Deutschland schon heute beim Anbau insgesamt als waldbaulich und ökonomisch betriebssichere Baumart (Wald und Holz NRW 2014a, b).

In Deutschland ist das Saatgut der slawonischen Stieleiche durch die „DKV - Gütegemeinschaft für forstliches Saat- und Pflanzgut e.V." als Sonderherkunft (SHK) ausgewiesen und zu beziehen. Laut DKV werden bei der slawonischen Stieleiche die Herkünfte „Münsterländer Späteiche - Slawonische Stieleiche", „Königsforst" sowie „Kottenforst" aus Nordrhein-Westfalen und die „Späteiche Burg Eltz" aus Rheinland-Pfalz aufgeführt (DKV 2021).

### 2.1.3 Eiche im Hinblick auf den Klimawandel

Seit vielen Jahren stehen der Klimawandel und die damit einhergehende globale Erderwärmung im Fokus verschiedenster Forschungsarbeiten. Schon lange prognostizieren Forscher anhand verschiedener Klimamodelle wärmere und trockenere Sommer sowie wärmere und feuchtere Winter (Wald und Holz NRW 2014b). Gegenwärtig lassen sich erste Auswirkungen des Klimawandels insbesondere in Waldökosystemen erkennen und bringen viele Fragen in Bezug auf die zukünftige Waldbewirtschaftung mit sich. Insbesondere aufgrund ihrer Langlebigkeit unterliegen Bäume einem stetigen Wandel und müssen sich an die laufenden Umweltveränderungen, vor allem aber an die durch Klimawandel bedingten höheren Temperaturen, die damit verbundenen längeren Vegetationsperioden und die geringeren Niederschläge, anpassen (Kölling und Zimmermann 2007, Pretzsch et al. 2014). Vorrausetzung sowohl für diese Anpassung als auch für den Erhalt der Anpassungsfähigkeit für zukünftige Generationen ist eine ausreichende genetische Variation der Populationen (Vornam et al. 2004, Gailing et al. 2008). Jedoch besitzen nicht alle Baumarten dieselben Anpassungspotentiale. So sind die in Deutschland forstwirtschaftlich wichtigsten Baumarten Buche und Fichte bereits heute anfällig gegenüber klimawandelbedingten höheren Temperaturen und einer abnehmenden Wasserkapazität im Boden (Kölling und Zimmermann 2007, Friedrichs et al. 2009, Kuster et al. 2011). Dementgegen steht die Eiche als generelle hitze- und trockentolerante Baumart, die durch ihre Pfahlwurzeln auch Wasser aus tieferen Bodenschichten erreichen kann, welches für andere Baumarten nicht mehr erschließbar ist (Leuschner et al. 2001, Kuster et al. 2011, Bonfils et al. 2015). In der Schweiz geht man laut Walthert et al. (2015) davon aus, dass sich ein Großteil der Waldstandorte bis in die Jahre 2070-2099 hin zu Kiefern- und Eichenstandorten verändern wird. Klimabasierte Modelle zeigen für Frankreich eine Verschiebung des Verbreitungsgebiets von

Baumarten und eine Ausdehnung des Eichenverbreitungsgebiets (vor allem der Steineiche) (Cheaib et al. 2012).

In Deutschland werden aktuell viele trockene Standorte mit Traubeneiche aufgeforstet, da sie ihr Potential unter wärmeren und trockeneren Bedingungen schon vielfach gezeigt hat. Es ist davon auszugehen, dass sich ihre Konkurrenzkraft während des Klimawandels noch weiter steigern wird (Wald und Holz NRW 2014a, b). Wie gut sich deutsche Stieleichenherkünfte hierzulande lokal an den Klimawandel anpassen können, muss erst noch in geeigneten Herkunftsversuchen untersucht werden. Sicher ist allerdings, dass die wuchsstarke Stieleiche auch das Potential besitzt, sich aufgrund ihrer hohen Plastizität sehr flexibel an ändernde Umweltveränderungen anzupassen (Bonfils et al. 2013). Problematisch für alle bisherig angebauten heimischen Eichenarten ist allerdings schon heute die Fülle an Schädlingen, die mit voranschreitendem Klimawandel noch stärker auftreten werden. Vor allem aus Waldzustandserhebungen, die immer öfter auf schlechte Kronenzustände der Eichenbestände hinweisen, wird deutlich, dass Gefährdungen durch Eichenfraßgesellschaften, Eichenprachtkäfer und Eichenkomplexerkrankung keinesfalls unterschätzt werden sollten (Niedersächsische Landesforsten 2021).

Hier werden einige Vorteile der Varietät slawonische Stieleiche deutlich. So gilt sie nicht als fremdländisch, wächst schnell mit guten Qualitäten, besitzt eine breite Standortamplitude und ist in Deutschland bei verschiedensten Mischungsanteilen bisher wenig bis gar nicht anfällig gegenüber den hier häufig auftretenden Krankheiten, wie Anpflanzungen seit 1870 deutlich zeigen (siehe Kapitel 1.2). Mittlerweile bestehen zudem einige „deutsche“ Bestände für Saatgutgewinnung und Züchtung. Außerdem eignen sich die vorhandenen genomischen Ressourcen langfristig für eine markergestützte Züchtung. Dennoch ist sie aufgrund ihres hohen Grundwasserbedarfs nicht für alle Wuchsgebiete gleichermaßen geeignet. Sofern es um die klimatische Anpassung im Hinblick auf den Klimawandel geht, dürfte eine Einbringung dieser Varietät langfristig unproblematisch möglich sein, da das Klima in ihrem Ursprungsgebiet Slawonien (1976-2018 Zagreb als nächstgelegene Wetterstation mit durchgängigen Aufnahmen: Jahresdurchschnittstemperatur: 12,3 °C, durchschnittliche Niederschlagsmenge: 867,2 mm [verändert nach Bronić et al. 2020]) schon jetzt klimatische Begebenheiten aufweist, die in Deutschland erst zukünftig (1961-2020: Jahresdurchschnittstemperatur: 8,8° C, durchschnittliche Niederschlagsmenge: 790,2 mm [Mühr 2021]) erwartet werden. Nach verschiedenen Szenarien von Jacob et al. (2008) mit niedrigen, mittleren und hohen Treibhausgaskonzentrationen wird demnach in Deutschland bis zum Jahr 2100 je nach Region maximal eine steigende Jahresmitteltemperatur zwischen 2,5°C und 3,5°C prognostiziert.

## 2.2 Bisherige Studien zur Untersuchung genetischer Variation der slawonischen Stieleiche

### 2.2.1 Untersuchungen anpassungsrelevanter genetischer Variation der slawonischen Stieleiche anhand von Herkunftsversuchen

Bis Anfang des 19. Jahrhunderts fungierten die slawonischen Stieleichenbestände vor allem als Pufferzone zwischen Österreich-Ungarn und dem Türkischen Reich und dienten in dieser Zeit als Hutewald nur einer landwirtschaftlichen, aber keiner forstlichen Bewirtschaftung (Klepac 1996). Das Erfassen der genetischen Variation gewann erst im 20. Jahrhundert an Bedeutung. Der Beginn der Forschung war bis in die 1920er Jahre von phänotypischen Untersuchungen in Herkunftsversuchen und natürlichen Stieleichenbeständen geprägt, die vornehmlich nur auf örtlicher Ebene von ambitionierten Förstern durchgeführt wurden (Wachter 2011). Herkunftsversuche zeichnen sich dadurch aus, dass Populationen unterschiedlicher geographischer Herkunft in einer gemeinsamen Umwelt (an einem Ort) getestet werden (Bogdan et al. 2017).

Der erste 1903 unter wissenschaftlichen Standards begründete Herkunftsversuch brachte 1923 von Cieslar (1923) veröffentlichte Erkenntnisse. Neben einigen heimischen Stieleichenbeständen wurden in diesem Versuch auch 3 Herkünfte aus der Save-Niederung (Lipovljani, Bos. Gradiska und Jamina) näher über phänotypische Merkmalsausprägungen untersucht. Durch Krieg, Reperationshiebe und den Bauboom änderte sich die Baumartenzusammensetzung stark hin zu schnellwachsenden, lukrativeren Baumarten für die Forstwirtschaft, sodass Herkunftsversuche der Eiche in den Hintergrund rückten. In ihrer kroatischen Heimat hingegen wurde die slawonische Stieleiche bei gleichzeitigem Rückgang ihrer natürlichen Anbaufläche aufgrund der Qualitäten und des Exportpotentials, forstwirtschaftlich interessanter (Rieger 2018). In jüngerer Vergangenheit setzten sich daher insbesondere in ihrem Ursprungsgebiet Herkunftsversuche als Forschungsansätze durch. Ein für Kroatien bedeutender Versuch wurde zwischen 1985-1986 an der Baumschule des Forschungsinstituts in Jaska (Kroatien) mit 6 slawonischen unter insgesamt 15 kroatischen und einer serbischen Stieleichenprovenienz angebaut (Gračan 1993). Das Ziel war, anhand der beobachteten Merkmale Wuchsleistung (Höhe) und Überleben Rückschlüsse auf die Holzproduktivität verschiedener Provenienzen in Kroatien zu ziehen. Bereits nach den ersten 5 Jahren zeigte die slawonische Provenienz Spačva eine auffallend starke Wuchsleistung. Bedingt durch die bisher allein auf phänotypischen Beobachtungen basierenden Erkenntnisse ist es problematisch, anhand dieser Ergebnisse auch etwaige Aussagen über die potenzielle Anpassungsfähigkeit an den prognostizierten Klimawandel (mit abnehmender Bodenwasserverfügbarkeit) zu tätigen. Aus Sicht der Forstgenetik reicht dafür das derzeitige Wissen sowohl über die adaptive genetische Variabilität als auch über die Differenzierung von Stieleichenbeständen südöstlich der Alpen insgesamt nicht aus (Slade et al. 2008).

Um hier entgegenzuwirken wurden zwischen 2008 und 2010 daher 3 weitere Feldversuche mit repräsentativeren Stichprobengrößen an Populationen in Slawonien etabliert. Einer dieser Versuche von Bodgan et al. (2017) in der Region Osijek-Baranja durchgeführt, beschäftigte sich explizit mit der potentiellen Anpassungsfähigkeit slawonischer Stieleichen an mögliche abnehmende Bodenwasserverfügbarkeiten. Für den Versuch wurden, neben 5 österreichischen, 17 kroatische Stieleichenpopulationen verschiedener Herkünfte, aus der Nähe der Grenze ihres südlichen Verbreitungsgebietes, in einen zusammenhängenden Waldkomplex (10.000 ha, überwiegend dominierenden Stieleichen) eingebracht (Bogdan et al. 2017). Über die Auswahl von Populationen am Rande ihres Verbreitungsgebietes erhoffte man sich Pflanzmaterial, das durch einwirkende Randeffekte z. B. des Klimas geprägt ist und damit insgesamt zu potenziell anpassungsfähigeren Varianten führt. Der sehr trockene Versuchsstandort zeichnete sich durch eine mittlere Lufttemperatur von 16,1 °C und einen mittleren Niederschlag von 562 mm während der Vegetationsperiode aus. Zusätzlich waren die Stieleichenpopulationen durch geringere Niederschläge einem erheblichen Rückgang der Wasserverfügbarkeit während der Vegetationsperiode und einem damit einhergehenden absinkenden Grundwasserspiegel ausgesetzt (Bogdan et al. 2017). Die Ergebnisse waren eindeutig und zeigten zwischen den Populationen signifikante Unterschiede im Überleben (visuell bewertet) und Höhenwachstum (in cm gemessen). Wie erwartet worden war, stammten die Populationen mit den höchsten Überlebensraten aus relativ trockeneren Lebensräumen Slawoniens. Dies lässt sich als eine lokale Anpassung an die abnehmende Wasserverfügbarkeit deuten (Bogdan et al. 2017).

Obwohl Herkunftsversuche bis in die Gegenwart innerhalb der Forschung genutzt werden, haben sie den großen Nachteil, dass sie relativ teuer sind und viel Zeit vergeht, bis Ergebnisse deutlich werden. Hier ergibt sich ein großer Vorteil der Umweltassoziationsanalysen entlang von Umweltgradienten mit Hilfe von SNP (‚Single Nucleotid Polymorphism') - Markern, die sich in jüngerer Vergangenheit in der forstgenetischen Forschung durchgesetzt haben und auf Einzelbaumebene darauf abzielen, genetische Varianten zu identifizieren, die direkt mit bestimmten Umweltfaktoren, z. B. Trockenstress, verbunden sind. Diese Gene sind Kandidaten für eine lokale Anpassung und werden ausgewählt, um neutrale und adaptive Diversitätsmuster neu zu berechnen. Aktuell untersuchten Temunović et al. (2020) über diesen Ansatz als erste die genetischen Strukturen der slawonischen Stieleiche. Ihre durchgeführten Umwelt-Assoziationsstudien zeigten konsistente Assoziationen zwischen SNP-Allelfrequenzen und verschiedenen klimatischen Variablen. Es konnten in 35 Genen insgesamt 37 SNP-Marker gefunden werden, die mit der Wasserverfügbarkeit assoziiert waren. Somit scheint sich zu bestätigen, dass trockenheitsbedingter Selektionsdruck die adaptive genetische Variation beeinflusst.

### 2.2.2 Untersuchungen räumlicher Strukturen der slawonischen Stieleiche im Ursprungsgebiet anhand von Chloroplastenmikrosatelliten

In jüngerer Vergangenheit halfen vor allem die voranschreitende Entwicklung molekularer Marker sowie genauere Erkenntnisse zur nacheiszeitlichen Rückwanderungsgeschichte, frühere Pflanzenverbreitungen noch besser zu rekonstruieren (Hewitt 1999, Petit et al. 2002a, Leinemann et al. 2018). Brewer et al. (2002) beschrieben auf Grundlage der verfügbaren Pollendaten die Refugien und nacheiszeitlichen Wiederbesiedlungsdynamiken der Gattung *Quercus* in Europa. Gleichzeitig wurden erstmals molekulare Marker für das Chloroplastengenom, welches bei Angiospermen in der Regel mütterlicherseits vererbt wird (Dumolin et al. 1995), eingesetzt, um die nacheiszeitlichen Rückwanderungswege besser zu verstehen (Petit et al. 2002a). Über diesen Ansatz konnten die Chloroplasten-Haplotypen (HP) 2, 4, 5, 6, 7-26, 17 und 31, die zu den Abstammungslinien A (HP4, HP5, HP6, HP7-26, HP31), C (HP2) und E (HP17) gehören, im Ursprungsgebiet der slawonischen Stieleiche identifiziert werden (Bordács et al. 2002, Petit et al. 2002a, b, Slade et al. 2008) (siehe Tab. 1).

Insgesamt kommen in Kroatien sieben verschiedene cpDNS-Haplotypen aus drei der sechs europäischen Abstammungslinien vor, woraus ersichtlich wird, dass die überlebenden Populationen aus verschiedenen postglazialen Refugien stammen. Hampe und Petit (2005) vermuten, dass aufgrund des von Petit et al. (2002b) angenommenen zweiten Refugiums der Stieleiche während der letzten Eiszeit im heutigen Kroatien die slawonische Stieleiche ein hohes Maß an adaptiver genetischer Variabilität aufweist.

**Tabelle 1** Überblick über die in Slawonien vorkommenden Haplotypen und deren Verbreitungsgebiete. Farben und Abstammungslinien stammen aus Petit et al. (2002b).

| Haplotyp (HP) | Abstammungslinie (Refugium) | Verbreitungsgebiet |
|---|---|---|
| HP2 | C (Apennin) | Von Kroatien bis nach Sizilien |
| HP4 | A (Balkan) | Zentraleuropa, aber fehlt im äußersten Osten und Westen Europas |
| HP5 | A (Balkan oder Apennin) | Von Italien bis in den Osten des Balkans, kommt auch in Deutschland natürlich vor |
| HP6 | A (Balkan) | Im Osten Europas mit dem südlichsten Vorkommen im Osten Kroatiens |
| HP7-26 | A (verschiedene eiszeitliche Refugien, vermutlich Pyrenäen oder Südalpen) | Von Norden Kroatiens bis in den Nordosten Kataloniens |
| HP17 | E (Apennin) | sehr weites Verbreitungsgebiet: Von Italien bis in den Osten Balkans, weiter bis in den Osten und Norden Kroatiens |
| HP31 | A (Balkan) | hauptsächlich in Rumänien |

### 2.2.3 Untersuchungen zur anpassungsrelevanten genetischen Variation und zur räumlichen Variation genetischer Strukturen in Deutschland

Veränderliche, Klimawandel bedingte Temperaturextreme führen wie schon heute häufig beobachtet wird zu einem erhöhtem Spätfrostrisiko, das insbesondere für Knospen und Blätter gefährlich ist (Liu et al. 2018, Vitasse et al. 2018). Der Zeitpunkt des Knospenaufbruchs wird als Merkmal für die Etablierung klimastabiler Bestände zukünftig eine zunehmend wichtigere Rolle spielen (Müller und Gailing 2019). In diesem Zusammenhang erlangte die slawonische Stieleiche in der deutschen Forstwirtschaft an Bekanntheit, da sie neben ihren bemerkenswerten ertragskundlichen Merkmalen (siehe Kapitel 1.2) primär durch ihren bis zu 3 Wochen späteren Blattaustrieb verglichen mit autochthonen Stieleichenbeständen in Deutschland auffiel (Wachter 2001). Der Austriebszeitpunkt gilt als adaptives Merkmal, das eine genetische Komponente besitzt und an die klimatischen Bedingungen des jeweiligen Ursprungsgebiets einer Population angepasst ist (Gailing et al. 2003, Schüler et al. 2012). Die Kenntnis der genetischen Variation von adaptiven Merkmalen ist in Bezug auf die Anpassungsfähigkeit an den Klimawandel und der damit einhergehenden Frage nach geeigneten Herkünften (Anbau von Herkünften aus „wärmeren“ Regionen) unabdingbar (Aitken et al. 2008, Savolainen et al. 2007). Im Jahr 2003 ist die Korrelation zwischen Austriebszeitpunkt und genetischer Variation in der Chloroplasten-DNS (cpDNS) an 3 einheimischen und 2 slawonischen Stieleichenbeständen in Hamm-Westtünnen (NRW) erstmals untersucht worden (Gailing et al. 2003). Insbesondere die Haplotypen 2 und 5, die der Ursprungsregion der slawonischen Stieleiche in Kroatien entstammen (siehe Kapitel 2.2, Tab. 1) und ihren Verbreitungsschwerpunkt auf dem Balkan haben, aber auch der Haplotyp 10 mit Vorkommen in Westeuropa und Verbreitungsschwerpunkt in Frankreich waren in dieser Studie mit einem späteren Austrieb assoziiert. Die durch einen frühen Austrieb charakterisierten einheimischen Bestände zeigten alle den Haplotyp 1, der in Deutschland am weitesten verbreitet ist. Es konnte eine starke Korrelation zwischen Haplotyp und Austrieb, welcher auf die jeweilige geografische Herkunft zurückzuführen ist, festgestellt werden (Gailing et al. 2003). Während kein kausaler Zusammenhang zwischen Haplotyp (cpDNS Variation) und Austrieb (anpassungsrelevanter Variation) besteht (Kremer et al. 2002), zeigten z.B. Individuen mit HP2, der ausschließlich auf dem Balkan vorkommt, einen deutlich späteren Austrieb als nördliche einheimische Provenienzen (Gailing et al. 2003).

Weitere Untersuchungen zur Charakterisierung verschiedener Ursprungsgebiete slawonischer Stieleichenbestände in Nordrhein-Westfalen (insbesondere Münsterland) fanden die slawonischen Haplotypen 2, 5, 7-26 und 17, wobei HP7-26 relativ selten auftrat (Gailing et al. 2007a, b, 2009). Von den 7 in Slawonien gefundenen Haplotypen (siehe Tab. 1), wurden 4 Haplotypen auch in Deutschland nachgewiesen. Dies deutet auf eine vergleichsweise hohe Variation der slawonischen Stieleichenbestände in Deutschland hin. Die in Deutschland häufigen Haplotypen 2 und 5, sowie der

vereinzelt auftretende HP17, lassen auf ein Ursprungsgebiet in den Niederungen zwischen den Flüssen Save und Drau in Kroatien schließen, wobei die genaue Herkunft bzw. geografische Lage der einzelnen Saatgutbestände nicht näher eingegrenzt werden kann (siehe Hesmer 1955, Gehle 1999). Zudem können auch mit den cpDNS-Untersuchungen keine Rückschlüsse auf eine Einfuhr genetischen Materials aus einem begrenzten Gebiet Slawoniens gezogen werden. Vielmehr scheinen die cpDNS-Untersuchungen darauf hinzudeuten, dass die „deutschen“ slawonischen Bestände aus unterschiedlichen Ursprungsregionen stammen und daher möglicherweise unterschiedliche Anpassungen aufweisen.

Mithilfe autochthoner Bestände aus 4 Regionen in NRW, die vor 1850 und vor dem Beginn des extensiven Samentransportes etabliert wurden, erhoffte man sich, den Einfluss natürlicher Prozesse wie anthropogener Samentransport und nacheiszeitliche Rückwanderung auf die genetische Variation an cpDNS-Markern noch besser analysieren und darstellen zu können. Hierbei wurden neben den erwarteten einheimischen Haplotypen 1, 10, 11 und 12, auch der südöstliche Haplotyp 7-26, nicht aber HP5, gefunden (Gailing et al. 2009). Dies könnte indirekt einen Hinweis darauf geben, dass der in anderen Beständen auftretende Haplotyp 5 einen slawonischen Ursprung haben könnte, da er in Beständen vor 1850 nicht auftritt. In einer aktuellen Studie konnten mithilfe von Kernmikrosatellitenmarkern Stieleichenbestände mit HP5 den in NRW wachsenden slawonischen Stieleichenbeständen zugeordnet werden (Burger et al. 2021). Hierbei zeigte sich jedoch, dass die Kombination der Ergebnisse beider Markerarten (Chloroplastenmarker (wie in Gailing et al. 2007a, b, 2009) und Kernmikrosatellitenmarker (wie in Burger et al. 2021)) notwendig ist, um sowohl zwischen sich nur geringfügig voneinander unterscheidenden Taxa zu differenzieren als auch um Beimischung zu erkennen. Insgesamt ist die neutrale genetische Differenzierung an den Kernmikrosatellitenmarkern vergleichsweise gering. Die Differenzierung an ertragssteigernden Merkmalen ist daher wahrscheinlich das Resultat genetischer Differenzierung an adaptiven Genen in Folge lokaler Anpassung. Beide Taxa (slawonisch und einheimisch) sind vermutlich nur an genomischen Regionen differenziert, die für diese Unterschiede verantwortlich sind, während das übrige Genom aufgrund von Genfluss und gemeinsamer Abstammung eine geringe Differenzierung aufweist. Zur Identifizierung adaptiver Unterschiede können beispielsweise QTL- Analysen und genomweite Vergleiche zwischen den Taxa („Outlier-Analysen“, z.B. Leroy et al. 2020) dienen.

### 2.2.4 Assoziationen zwischen genetischer Variation und Merkmalsvariation (QTL) der innerartlichen Kreuzungsnachkommenschaft *Q. robur* subsp. *robur* und *Q. robur* subsp. *slavonica*

Zahlreiche relevante Merkmale in der Forstwirtschaft, wie beispielsweise Wuchshöhe oder Trockenheitstoleranz, werden nicht nur von einem Gen beeinflusst und vererbt, sondern sind von einer Vielzahl genetischer Faktoren und Umwelteinflüssen abhängig. Man spricht daher von quantitativen Merkmalen, für deren Ausprägung mehrere Abschnitte eines Genoms (QTL, Quantitative Trait Loci) verantwortlich sind. In der Forstgenetik und Forstpflanzenzüchtung werden QTL-Kartierungen dazu verwendet, diese chromosomalen Bereiche und die darin befindlichen DNS-Marker zu lokalisieren, die mit der Ausprägung des untersuchten phänotypischen Merkmals korreliert sind (Finkeldey 2010, Gonzalez-Martinez et al. 2006). Mithilfe der QTL-Kartierungen lassen sich anpassungsrelevante Gene grob lokalisieren und deren Einfluss auf adaptive Merkmalsvariation ermitteln, allerdings befinden sich in dem chromosomalen Bereich, in dem ein QTL nachgewiesen wird, mehrere hunderte Gene, sodass diese weder präzise erkannt noch funktional charakterisiert werden (Finkeldey und Hattemer 2010). Diese anpassungsrelevanten Gene könnten in genomweiten Assoziationsanalysen (GWAS) validiert werden und der markergestützten Selektion von Individuen sowie der Abschätzung der Anfälligkeit gegenüber biotischem und abiotischem Stress in Hinblick auf den Klimawandel dienen (z.B. Rellstab et al. 2021). QTL-Kartierungen sind dennoch wichtige Verfahren, um die genetische Grundlage quantitativer morphologischer Merkmale innerhalb und zwischen Arten aufzudecken (Gailing 2008). Vor allem im Hinblick auf den Klimawandel ist die Kenntnis genetischer Variation für Anpassungsprozesse und ertragsbestimmende Merkmale von großem Interesse (Schüler et al. 2012). Für Bäume konnten auf diese Weise schon verschiedene Merkmale, z. B. für Holzqualität, Laubaustieb, Trockenheitstoleranz sowie Winterhärte, festgestellt werden (Krutovsky & Neale 2005). QTL-Kartierungen werden in Vollgeschwisterfamilien durchgeführt, da nach kontrollierter Kreuzung nicht nur ein einzelner Marker mit einem Merkmal assoziiert ist, sondern aufgrund genetischer Kopplungen auch eine Assoziation mit den angrenzenden Markern einhergeht (Gailing 2010). Mit diesem Ansatz war es in bisherigen Studien möglich, anhand einer kontrollierten Kreuzungsnachkommenschaft zwischen *Q. robur* x *Q. robur* subsp. *slavonica* (387 Vollgeschwister) QTLs für artdifferenzierende blattmorphologische Merkmale sowie für potentiell adaptive Merkmale (Stomatadichte, Blattaustrieb, Wassernutzungseffizienz) zu identifizieren (siehe Gailing 2008, Gailing et al. 2008, Gailing et al. 2013). Dabei zeigten Stomatadichte und Wachstum eine positive Korrelation unter kontrollierten Gewächshausbedingungen. Allerdings konnten die in den genomischen Regionen enthaltenen Gene bis heute nicht direkt identifiziert werden.

## 2.3 Schlussfolgerung und Ausblick

Dass ein Waldumbau hin zu klimastabileren Wäldern zwingend notwendig sein würde, hat sich seit einigen Jahrzehnten abgezeichnet. Die Geschwindigkeit, mit der dieser Umbau nun umgesetzt werden muss, ist nicht zuletzt den teils schweren Kalamitäten der letzten Jahre geschuldet. Insbesondere Spätfrostrisiko, steigende mittlere Jahrestemperaturen, häufiger auftretende Trockenperioden und längere Vegetationsperioden infolge des Klimawandels führen dazu, dass Bäume sich den laufenden Umweltveränderungen schneller anpassen (Kölling und Zimmermann 2007, Pretzsch et al. 2014) und Wälder mit Arten einer breiteren Standortamplitude bestockt werden müssen. Sowohl als Nebenbaumart zur Stabilität in Mischbeständen aber auch in Form von Reinbeständen bekommen Eichenarten einen besonderen Zuspruch in der Forstwirtschaft. Die slawonische Stieleiche ist aufgrund ihrer positiven Eigenschaften eine der Hoffnungsträger und wird als möglicher Ersatz für heimische Stieleichenpopulationen diskutiert. Sie gilt in einem Großteil der Wuchsgebiete Deutschlands als standortgerecht, da ihre ökologischen Ansprüche an klimatische Begebenheiten mit den tatsächlichen Wuchsbedingungen übereinstimmen. Darüber hinaus charakterisieren aktuelle Studien (z.B. Burger et al. 2021) die slawonische Stieleiche als Varietät und nicht als Unterart. Damit nimmt sie eine Sonderstellung ein und gilt im Hinblick auf den Klimawandel nicht als neu eingebrachte Art, sondern als eine alternative Herkunft. Die genetischen Unterschiede zu unserer heimischen Stieleiche sind mit Ausnahme von anpassungsrelevanten Unterschieden gering. Eine der wichtigsten Vorrausetzungen sowohl für Anpassung als auch für den Erhalt der Anpassungsfähigkeit in zukünftigen Generationen bildet eine ausreichende genetische Variation von Populationen (Vornam et al. 2004, Gailing et al. 2008). Während viele Untersuchungsansätze der Vergangenheit auf morphologischen Betrachtungen oder Schätzungen fußen und als relativ langwierig in der Umsetzung gelten, ermöglicht die Entwicklung neuer Marker eine sehr genaue Analyse der genetischen Variation von Baumarten. Molekulare Marker wie beispielsweise Chloroplasten-DNS Marker werden als kostengünstig sowie effizient eingestuft und ermöglichen schnelle und sichere Untersuchungen der Haplotypenvariation, die z. B. zur Identifizierung postglazialer Differenzierungsmuster genutzt werden (Hewitt 1999). Petit et al. (2002b) sowie Hampe und Petit (2005) konnten über diese Marker nachweisen, dass die überlebenden Populationen aus verschiedenen postglazialen Refugien stammen müssen und dass dies vermutlich ursächlich für das hohe Maß an adaptiver genetischer Variabilität der slawonischen Stieleiche im heutigen Kroatien ist.

Für die weitere Etablierung der slawonischen Stieleiche in Deutschland ist es elementar, diejenigen Gene zu identifizieren, die wesentlich zur Variation von Merkmalen innerhalb der Art beitragen. Es konnten zwar bisher Assoziationen zwischen der Variation in genomischen Regionen auf Kopplungskarten und Merkmalen (QTLs) gefunden werden (Gailing et al. 2008, 2013), allerdings gelang

es nicht, die in den genomischen Regionen enthaltenen Gene zu identifizieren. Hier sollte zukünftig versucht werden mithilfe von „Next Generation Sequencing“ QTL Regionen und gekoppelte Gene, die an der Merkmalsausprägung beteiligt sind, zu lokalisieren und über genomweite Assoziationsstudien, z.B. in Herkunftsversuchen, Kandidatengene für anpassungsrelevante Variation zur Charakterisierung des Anpassungspotentials zu finden. Weiterhin sollte über Ausreißertests versucht werden genetische Varianten zu identifizieren, die eine höhere oder niedrigere Differenzierung zwischen Populationen aufweisen als dies unter neutralen Annahmen erwartet würde. Höhere Differenzierung kann ein Zeichen von gerichteter Selektion sein, während niedrigere Differenzierung als balancierende Selektion interpretiert wird. Damit könnten langfristig nicht nur offene Fragen zur genomweiten Differenzierung zwischen den Varietäten (slawonische und einheimische Stieleiche) geklärt, sondern auch die forstliche Praxis bei Züchtungsprogrammen oder der Etablierung von Samenplantagen unterstützt werden.

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Kapitel 3

# Genetic differentiation of indigenous (*Quercus robur* L.) and late flushing oak stands (*Q. robur* L. subsp. *slavonica* (Gáyer) Mátyás) in western Germany (North Rhine-Westphalia)

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# Genetic differentiation of indigenous (*Quercus robur* L.) and late flushing oak stands (*Q. robur* L. subsp. *slavonica* (Gáyer) Mátyás) in western Germany (North Rhine-Westphalia)

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**Author contributions** KB conducted the laboratory work and data analysis, prepared the first draft of the manuscript including the preparation of all figures and tables, and contributed to the revision and interpretation of the results. MR provided all information on the individual stands in NRW. MM, MR and OG contributed to the interpretation of the results, reviewing and editing the manuscript. All authors contributed to the article and approved the submitted version.

## Abstract

Slavonian oaks (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) originating from Croatia have been cultivated in Germany mainly in the Münsterland region of North Rhine-Westphalia since the second half of the 19$^{th}$ century. Compared to indigenous pedunculate oak stands in Germany, they are characterised by their late bud burst, but also by their excellent bole shape and faster height growth. Previously, Slavonian pedunculate oaks (=late flushing oaks) were mainly studied at chloroplast (cp) DNA markers in order to determine their geographical origin. The origin of the material is probably the Sava lowland between Zagreb and Belgrade. In the present study, the aim was to genetically differentiate between indigenous *Quercus robur* and Slavonian oak stands using nuclear DNA markers. For this purpose, we used 20 nuclear Simple Sequence Repeats (nSSRs). A total of 37 pedunculate oak stands (mean: 18.6 samples per population with an age of 95 to 210 years) were examined, of which 21 were characterized as Slavonian late flushing oaks and three stands for which the Slavonian origin was not clear. Maternally inherited chloroplast markers were analysed earlier in all 37 stands to validate their geographic origin. We found that the stands of native pedunculate oaks and Slavonian pedunculate oaks are represented by two genetic clusters which are weakly differentiated. Slavonian oaks ($N_a$=9.85, $A_r$=8.689, $H_o$=0.490, $H_e$=0.540) showed similar levels of genetic variation as native oak stands ($N_a$=7.850, $A_r$=7.846, $H_o$=0.484, $H_e$=0.526). Differences in growth and phenology and low but consistent genetic differentiation between groups suggest that both taxa represent different ecotypes with specific local adaptations, which are perhaps separated by less overlapping flowering phenologies. The nuclear microsatellite markers in combination with the cpDNA markers are suitable to differentiate between Slavonian and local oak stands.


**Key words:** Slavonian oak, microsatellites, identification of origin, genetic assignment

## 3.1 Introduction

The Slavonian pedunculate oak (*Quercus robur* L. subsp. *slavonica* (Gáyer) Mátyás) is an established naturalized variety of the native pedunculate oak (*Quercus robur* L.) in Germany and occupies a special position within this species. Slavonian oaks have been introduced into the western part of Germany, especially in the region around Münster, in the second half of the 19$^{th}$ century with the beginning of extensive seed trade through steam engines (Wachter 2001; Gailing et al. 2007a). According to historical documents and analysis with cpDNA markers, they have their geographic origin in the forest areas of the lowlands of the rivers Sava and Drava between Zagreb and Belgrade in the eastern region of Croatia (Wachter 2001; Gailing et al. 2007a, b). In Germany, Slavonian oaks are characterized by their late bud burst compared to indigenous oaks and are therefore significantly less affected by the European oak leaf roller (*Tortrix viridana*) and late spring frost (Wachter 2001). From a yield point of view, they have a high growth rate compared to indigenous pedunculate oaks and are characterized by their straight and long stem as well as by their fine branches (Wachter 2001; Gailing et al. 2003). The Slavonian oak stands are first generation stands in Germany which were established between 1870 and 1912 from seeds collected in the Eastern part of today's Croatia. A genetic characterization of putative Slavonian stands is important in order to identify seed production areas, certify reproductive material, identify mixed stands and detect gene flow between both taxa and later generation stands.

In previous studies (Petit et al. 2002 and Bordács et al. 2002) on the post-glacial recolonisation of pedunculate oak species in Europe and especially the Balkan region, haplotypes HP2, HP5, HP6, HP7-26 and HP17 were found in the area of origin of the Slavonian pedunculate oak (Croatia) and a glacial refugium of populations with these haplotypes in the Balkan region was suggested (Petit et al. 2002 and Bordács et al. 2002). Furthermore, in cpDNA marker studies of Slavonian oak populations in western Germany, the haplotypes HP2, HP5 and HP17 were found to be common Slavonian haplotypes (Gailing et al. 2003, Gailing et al. 2007a, b, 2009). However, only haplotype 2 does not occur naturally in Germany (Petit et al. 2002).

Expressed Sequence Tag (EST)-SSR markers (Durand et al. 2010; Burger et al. 2018; Müller and Gailing 2018) used are located in expressed genes and can be derived from publicly available genomic resources, so-called EST libraries, and are located either in coding regions or in 5‘ or 3‘ untranslated regions (UTRs) (Ellis and Burge 2007). In addition, they can be transferred across taxonomic boundaries because of their location in regions of the DNA that are strongly conserved within phylogenetically related species (Ellis and Burke 2007; Burger et al. 2018). Microsatellites, especially EST-SSRs, are important genetic markers widely used in population genetic analysis of forest tree species, including oaks (Streiff et al. 1998; Dzialuk et al. 2005; Ellis and Burke 2007; Lind and Gailing 2013; Sullivan et al. 2013; Müller and Gailing 2018). The genetic structure of a population is characterized by the number of subpopulations in it, the frequency of alleles in each subpopulation and the degree of genetic

isolation of the subpopulation (Chakraborty 1993). Population genetic structure can be analyzed through *F-statistics* (Wright 1965) and/or analysis of molecular variance (AMOVA) (Excoffier et al. 1992) or inferred by clustering individuals into groups (Greenbaum et al. 2016). Clustering of individuals into subpopulations based on genetic data from microsatellite analysis is an often used method (Greenbaum et al. 2016). Cluster analyses can be divided into two methods: I) Model-based approaches as implemented in the program STRUCTURE and II) distance-based approaches like principal coordinate analysis (Pritchard et al. 2000; Alexander et al. 2009; Greenbaum et al. 2016).

Based on these kind of analyses, the aim of the study is to distinguish genetically between indigenous oak stands and stands known as late-flushing oaks.

The research questions of our study were: (a) are the stands known as late flushing oak stands genetically differentiated from the indigenous oak stands in North Rhine-Westphalia? (b) Does the amount of genetic variation vary among varieties and/or are there indications of losses of genetic variation due to bottleneck effects in Slavonian oaks in North Rhine-Westphalia? (c) Are nuclear markers better suited than cpDNA markers to differentiate between indigenous and late flushing oak stands?

## 3.2 Materials and Methods

### 3.2.1 Plant material

For genotyping Slavonian pedunculate oak and common pedunculate oak populations with nuclear microsatellite markers, extracted DNA samples from 2005, 2006 and 2007 were used (Gailing et al. 2007a, b, 2009) (Table 1). The trees originate from 36 different populations from seven separate regions in North Rhine-Westphalia (Germany) (Fig. 1): the Minden Land (stands planted in 1894), Münsterland (planted between 1826 and 1890), Lower Rhine region (planted in 1878), Lower Rhine bay (planted between 1893 and 1912), Bergisch region (planted between 1887 and 1891) and Sauerland (planted in 1819) ( Table 1). Seeds for the establishment of the stand 28 (planted in 2007) were collected directly in Croatia (Vinkovski) (Gailing et al. 2007b). For each of the 37 populations, 16 to 20 samples were used for the genetic characterization with 20 nuclear microsatellite markers. The population Kottenforst 154B, however, consisted of only 4 samples (Table 1).

All trees of the stands (20 trees per stand) from Gailing et al. (2007a) are phenotypically (straight long bole, fast growth) as well as phenologically (late flushing) characterized as of Slavonian origin showing, based on a combination of PCR-RFLPs and cpSSRs, either haplotype 2 or haplotype 5, both of which are frequent in the Balkan region, but only haplotype 2 does not occur naturally in Germany (Gailing et al. 2007a). Most trees (20 trees per stand) from Gailing et al. (2007b) were also characterized as Slavonian pedunculate oaks due to their growth behaviour, late flushing and historical documents before characterized at cpDNA markers (Gailing et al. 2007b).

**Table 1** Overview of the Slavonian and indigenous oaks stands

| Region | population No. | stand | Haplotype[1)] | latitude | longitude | planting [year] | area [ha] | references |
|---|---|---|---|---|---|---|---|---|
| Münsterland | SL_19 | Warendorf-Ostenfelde, Frhr. v. Nagel-Doornick 33C[a] | 5 | 51°31'43.26" | 8°04'54.81" | 1878 | 1.8 | Gailing et al. 2007a |
| Münsterland | SL_15 | Warendorf-Ostenfelde, Frhr. v. Nagel-Doornick 36E[a] | 2 | 51°33'43.26" | 8°02'54.81" | ~1882 | 1.5 | Gailing et al. 2007a |
| Münsterland | SL_14 | Warendorf-Ostenfelde, Frhr. v. Nagel-Doornick 50B[a] | 5 | 51°55'43.26" | 8°03'54.81" | ~1883 | 1.6 | Gailing et al. 2007a |
| Münsterland | SL_16 | Warendorf-Westkirchen, Schulze-Sutthoff Flur 2/70[a] | 5 | 51°54'43.26" | 8°02'54.81" | ~1890 | 0.6 | Gailing et al. 2007a |
| Münsterland/Ruhr region | SL_17 | Hamm- Osttünnen 1B1a[a] | 2 | 51°38'43.26" | 7°54'54.81" | ~1894 | 0.4 | Gailing et al. 2007a |
| Münsterland/Ruhr region | SL_18 | Hamm- Osttünnen 1B1b[a] | 5 | 51°38'43.26" | 7°54'54.81" | ~1890 | 0.6 | Gailing et al. 2007a |
| Bergisch region | SL_20 | form. FA Bergisch Gladbach, Königsforst 127c[a] | 5 | 50°57'21.44'' | 7°09'27.92'' | 1887 | 3.4 | Gailing et al. 2007b |
| Bergisch region | SL_23 | form. FA Bergisch Gladbach, Königsforst 76B[a] | 2 | 50°56'21.44'' | 7°10'27.92'' | 1891 | 5 | Gailing et al. 2007b |
| Croatia | SL_28 | nursery Jungermann, origin Croatia, Vinkovsi[a] | 5 | 45° 8' 43.26" | 18° 53' 54.81" | 2007 | - | Gailing et al. 2007b |
| Lower Rhine bay | DE_8 | form. FA Bonn, Kottenforst 134A&C | 1,4&**10** | 50°40'42.81'' | 7°03'56.29'' | ~1902 | 6.1 | Gailing et al. 2007b |
| Lower Rhine bay | SL_37 | form. FA Bonn, Kottenforst 154B[a] | **5** & **17** | 50°40'21.44'' | 7°00'27.92'' | 1912 | 3.2 | Gailing et al. 2007b |
| Lower Rhine bay | DE_9 | form. FA Bonn, Kottenforst 40B | 10 | 50°38'42.81'' | 7°04'56.29'' | 1907 | 2.2 | Gailing et al. 2007b |
| Lower Rhine bay | SL_22 | form. FA Bonn, Kottenforst 70D[a] | 5 & **17** | 50°39'21.44'' | 7°04'27.92'' | 1903 | 2.5 | Gailing et al. 2007b |
| Lower Rhine bay | DE_7 | form. FA Bonn, Kottenforst 85B | **1**, 4& 10 | 50°39'21.44'' | 7°02'27.92'' | 1905 | 4.1 | Gailing et al. 2007b |
| Lower Rhine bay | DE_6 | form. FA Bonn, Kottenforst 85D | 10 | 50°39'21.44'' | 7°02'27.92'' | 1904 | 8.6 | Gailing et al. 2007b |
| Lower Rhine bay / Voreifel | SL_21 | form. FA Bonn, Kottenforst, Tomberg 10B2[b] | 5 | 50°35'21.44'' | 6°58'27.92'' | 1893 | 1.5 | Gailing et al. 2007b |
| Lower Rhine region | SL_29 | Frhr. v. der Leyen 17C [b] | 5 | 51°16'42.81'' | 6°39'56.29'' | 1878 | 1.3 | Gailing et al. 2007b |
| Lower Rhine region | SL_24 | Stadt Viersen 36B/38[b] | 5 | 51°17'21.44'' | 6°20'27.92'' | 1886 | 16.7 | Gailing et al. 2007b |
| Lower Rhine region | SL_35 | Frhr. v. Nagel-Doornick, Steprath 3H[b] | 2 | 51°30'42.81'' | 6°13'56.29'' | 1881 | 0.5 | Gailing et al. 2007b |
| Minden Land | DE_11 | Gut Ulenburg 4C | 1 | 52°13'43.26" | 8°40'54.81" | 1894 | 1.6 | Gailing et al. 2007b |
| Münsterland | DE_12 | Fürst zu Salm-Salm, Rhede 1054 A1/A7 | 1 | 51°50'43.26" | 6°26'54.81" | 1885 | 2,3 | Gailing et al. 2007b |
| Münsterland | SL_31 | form. FA Warendorf, Schulze Pellengahr 4H[a] | 5 | 51°46'43.26" | 7°59'54.81" | 1893 | 1.2 | Gailing et al. 2007b |
| Münsterland | SL_36 | form. FA Letmathe, Estermann 116 A1[a] | 2 | 51°38'43.26" | 7°49'54.81" | 1894 | 2.2 | Gailing et al. 2007b |
| Münsterland | SL_32 | form. FA Letmathe, Graf v. Kanitz 19 A[a] | 5 | 51°39'43.26" | 7°31'54.81" | 1883 | 1.6 | Gailing et al. 2007b |
| Münsterland | SL_33 | form. FA Letmathe, Graf v. Kanitz 32H/39A[b] | 5 | 51°39'43.26" | 7°33'54.81" | 1862-1882 | 2 | Gailing et al. 2007b |
| Münsterland | DE_10 | form. FA Letmathe, Graf v. Kanitz 76 A | 1 | 51°41'43.26" | 7°34'54.81" | 1826-1864 | 9.5 | Gailing et al. 2007b |
| Münsterland | SL_26 | form. FA Letmathe, Graf v. Kanitz 77C[a] | 5 | 51°41'43.26" | 7°34'54.81" | 1883 | 4.4 | Gailing et al. 2007b |
| Münsterland | SL_25 | form. FA Letmathe, H. Blix, Cappenberg , Flur 2/155[a] | 5 | 51°41'43.26" | 7°42'54.81" | 1888 | 1 | Gailing et al. 2007b |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Münsterland | SL_27 | form. FA Letmathe, Schulze-Becking 54B1/B2[a] | 5 | 51°41'43.26" | 7°39'54.81" | 1880 | 1.5 | Gailing et al. 2007b |
| Münsterland | SL_34 | form. FA Obereimer, Graf v. Plettenberg 104 A[a] | 5 | 51°41'43.26" | 8°06'54.81" | 1895 | 1.8 | Gailing et al. 2007b |
| Münsterland | SL_30 | form. FA Obereimer, Graf v. Plettenberg 106G[a] | 5 | 51°41'43.26" | 8°05'54.81" | 1888 | 2.9 | Gailing et al. 2007b |
| Münsterland | SL_13 | form. FA Warstein-Rüthen, Kirche Anröchte 32C | 7-26 | 51°32'43.26" | 8°20'54.81" | 1894 | 4.2 | Gailing et al. 2007b |
| Münsterland | DE_2 | Studienfond Münster 11C | 1 | 51°59'43.26" | 8°06'54.81" | 1817 | 3 | Gailing et al. 2009 |
| Münsterland | DE_5 | Warendorf, Schulze Pellengahr 2A | 12 | 51°49'43.26" | 7°37'54.81" | 1800 | 4 | Gailing et al. 2009 |
| Münsterland | DE_3 | Graf Westerholt, Freckenh. 308B/309A | 1 | 51°52'43.26" | 7°51'54.81" | 1844 | 6.4 | Gailing et al. 2009 |
| Münsterland | DE_4 | Graf Merveldt 3A | 1 | 51°41'43.26" | 7°39'54.81" | ~1871 | 2.5 | Gailing et al. 2009 |
| Sauerland | DE_1 | form. FA Arnsberg 336B | 1 | 51°21'43.26" | 7°59'54.81" | 1819 | 5.4 | Gailing et al. 2009 |

[a] Described as Slavonian oaks (SL_XX) according to phenology (late flushing), growth habit, phenotype and/or historical documents; [b] Slavonian origin unclear; no letter= indigenous stands (DE_XX)
[1)]predominant haplotypes in bold face.

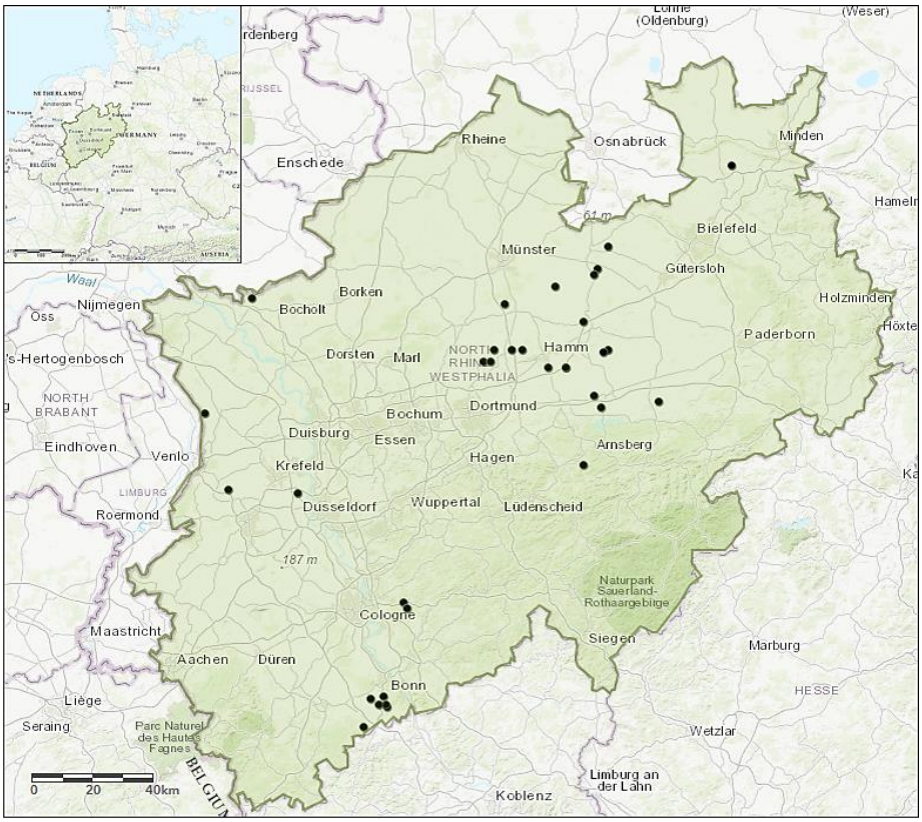


Figure 1 Geographical location of the sampled populations (black dots) in North Rhine-Westphalia. Map created in ArcGIS online (Esri, California, USA).

The predominant haplotypes in most populations are haplotype 2 and haplotype 5 (Table S. 1). In addition, the seeds collected in Vinkovsi (Croatia) for the establishment of stand 28 show haplotype 5 (Gailing et al. 2007b). Besides the Slavonian stands, indigenous stands were also selected in order to be able to compare these with each other. Therefore, populations (mean 18.2 trees per stand) were also selected from Gailing et al. (2007b) defined as common pedunculated oaks (haplotypes 1, 4 and 10). The remaining DNA samples representing indigenous oaks were taken from Gailing et al. (2009). These stands were all established before 1850 and were owned by smallholders, indicating that native plant material was used (Gailing et al. 2009). Haplotype 1 was the most common haplotype in indigenous stands and haplotype 12 occurred in only one population. Table S. 1 and Figure 2 give an overview on relative haplotype frequencies and distribution for each population in North Rhine-Westphalia.

Some Slavonian oak stands, such as Nagel-Doornick 50B, Hamm-Osttünnen 1B1b, Tomberg 10B2, Stadt Viersen 36B/38, Freiherr von der Leyen 17C, Plettenberg 104G, Steprath 3H and Estermann 116A1, also have a low relative frequency (0.05-0.125) of indigenous haplotypes (HP1 and HP10) (see Table S.1, Fig. 2). Conversely, the two indigenous oak stands, Kottenforst 134A&C and Gut Ulenburg 4C, also show the Slavonian haplotype 2 with a relative frequency of 0.05-0.1. The other 27 populations have either only indigenous haplotypes (HP 1, 4, 10, 12) or only haplotypes which are characteristic of Slavonian oaks (HP 2, 5, 7-26) (Table S.1, Fig. 2).

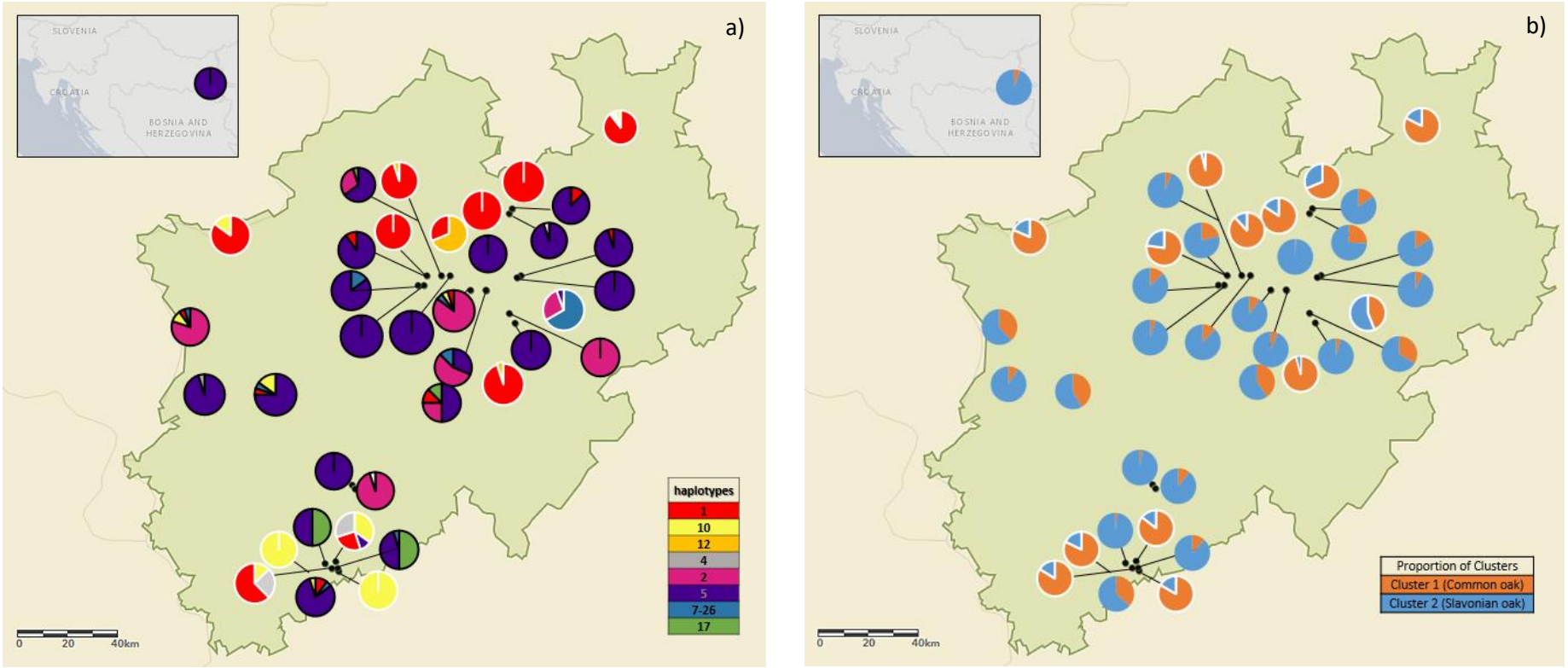


**Figure 2** a) Distribution of *Quercus robur* and *Quercus robur* subsp. *slavonica* (with black circle) chloroplast haplotypes in North Rhine-Westphalia. Populations' haplotypes were identified in earlier studies (Gailing et al. 2007a, b, 2009). HP1: Italy-Scandinavia line (lineage C), HP2: Croatia-Sicily line (lineage C), HP4: central Europe line (lineage A), HP5: Italy-Eastern Balkan-Germany line (lineage A), HP7-26: Croatia- Catalonia line (lineage A), HP10: Western Europe-Portugal line (lineage B), HP11, 12: Western Europe line (lineage B), HP17: Italy-Balkan line (lineage E) (as decsribed in Petit et al. 2002). b) Distribution of *Quercus robur* (with white circle) and *Quercus robur* subsp. *slavonica* proportion in STRUCTURE clusters in North Rhine-Westphalia. A higher proportion of ancestry in cluster 1=common oaks, a higher proportion in cluster 2=Slavonian oaks. The small section on the upper left shows the location of the stand Vinkovsi in Croatia. Maps generated with ArcGIS online.

### 3.2.2 Microsatellite analysis

A total of three genomic simple sequence repeats (gSSRs) (Sullivan et al. 2013) and 17 gene-based expressed sequence tag–simple sequence repeats (EST-SSRs) were used (Table 2). Due to the high transferability of EST-SSRs among species in *Quercus* and the availability of a large number of nSSRs and EST-SSRs developed for *Q. robur*, *Q. petraea* and related species (Steinkellner et al. 1997; Barreneche et al. 2004; Ellis and Burke 2007; Durand et al. 2010), we selected these 20 markers with reliable amplification in multiplex reactions. Thereof, 10 EST-SSR primer pairs were originally developed for *Quercus robur* L. (Durand et al. 2010). Seven EST-SSRs (Qr0057, Qr0332, Qr1423, FS_C2361, FS_C2660, FS_C2791 and FS_C8183) originally developed in *Q. rubra* were tested successfully in Müller and Gailing (2018) for their transferability to *Q. robur* based on primer sequences published by the 'Hardwood Genomics Project' (https://www.hardwoodgenomics.org/Transcriptome assembly/1963023?tripal_pane=group_description_download). The annotation of the sequences was obtained by searching the individual primer sequences in the respective contigs to identify the complete contig sequences for similarity searches against the UniProt Viridiplantae database (The UniProt Consortium 2017) using BLASTx (Basic Local Alignment Search Tool) (Altschul et al. 1990).

Table 2 Primer sequences and descriptions.

| developed for | Primer name | repeat motif | LG | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') | observed size in (bp) | Annotation of sequences |
|---|---|---|---|---|---|---|---|
| *Quercus rubra*[a] | 2P24[a] | $(CA)_{14}$ | - | GCAAGAGATCACACACAAACTAGC | CTTTGGGTTCACCAAACAGC | 131-162 | - |
| *Quercus rubra*[a] | 3A05[a] | $(CA)_{12}(CT)_2$ | - | AACGTGACCTCTCTCACAGC | AGTGCTGGAGTGCTCATGG | 139-162 | - |
| *Quercus rubra*[a] | 3D15[a] | $(CA)_{15}$ | - | GGTGGTGGCAGATACACTGG | GACTCAGACAACCAACTTCAGG | 210-217 | - |
| *Quercus robur* | FIR013 | $(CAG)_5$ | 2 | CGGGGAGGTTGATGAGTATT | AACACTGTCACCCCCATAGC | 129-150 | Constans 1 |
| *Quercus robur* | FIR028 | $(TC)_8$ | 1 | GGAAGAGTGTTCGGAAAGCA | CCAGCTCCTCCACAATAGCA | 199-215 | tropinone reductase homolog at1g07440 |
| *Quercus robur* | FIR035 | $(AT)_6$ | 5 | GCTAAGGTTCCGTGTTCCAA | GGCCAGCAACTAAACCAAGA | 146-177 | chaperone protein dnaj |
| *Quercus robur* | PIE125 | $(GGAAGC)_3$ | 6 | AATACAAATCGCAGGAGGTG | CTAACCCATCGTTCATGGAG | 139-180 | dnaj-like protein |
| *Quercus robur* | GOT040 | $(GA)_{11}$ | 6 | AAGGCACTCGTCGCTTTCTA | ACCGATTTGAAGCTCGAGAA | 210-249 | 40s ribosomal protein s16 |
| *Quercus robur* | VIT107 | $(TA)_{13}$ | 3 | TGATCACAGATTGGAGCTTAACA | CCCCCACTTAGGAAAGAAGC | 121-161 | light-harvesting complex i protein lhca2 |
| *Quercus robur* | VIT023 | $(ATA)_6$ | 5 | AATGCGAACGACATGAACAA | CTCTCGTCGGAGACTCAACC | 117-133 | ap2 erf domain-containing transcription factor |
| *Quercus robur* | FIR104 | $(GGT)_7$ | 11 | TTAACTCGGTTTGCGACTCA | AGCACGTGACTCGACCTGTA | 198-221 | r2r3-myb transcription factor |
| *Quercus robur* | PIE102 | $(AG)_{12}$ | 11 | ACCTTCCATGCTCAAAGATG | GCTGGTGATACAAGTGTTTGG | 138-167 | TC58546 similar to UP\|Q8NQE3 CORGL Predicted transcriptional regulator, partial |
| *Quercus robur* | PIE267 | $(AG)_{11}$ | 3 | CCAACCATCAAGGCCATTAC | GTGCGAACAGATCCCTTGTC | 74-114 | - |
| *Quercus rubra*[b] | Qr0057 | $(AAG)_7$ | - | CCGACCTTGTTGATTGTTCC | TATTGATCCTATCGGAGGCG | 125-153 | Glycoprotein gp2 |
| *Quercus rubra*[b] | Qr0332 | $(CCT)_5$ | - | AATATCAAATCGGCCAGCAG | GTGGTGGACCTGTGCCATAC | 150-175 | Keratin, ultra-high-sulfur matrix protein |
| *Quercus rubra*[b] | Qr1423 | $(CAC)_6$ | - | TCCCTTCTCGTTTCACCATC | TGCACCATACGGATTGAAAG | 261-303 | Epstein-Barr nuclear antigen 1 |
| *Quercus rubra*[c] | FS_C2361 | $(GAA)_8$ | - | AGGTCCTTCAGTTTGGGAGC | ATTCCCATGCATCAAAATCC | 185-240 | One-helix protein 2 |
| *Quercus rubra*[c] | FS_C2660 | $(GAG)_8$ | - | AGCAGAATTCGCCAAGTGAT | TGCCTTTGCATTCTCCTCTT | 218-240 | Uncharacterized protein |
| *Quercus rubra*[c] | FS_C2791 | $(GA)_5$ | - | CGAAACAGAGAGAACCCAAGA | CTTCAAACATCCAGCGTTGA | 287-298 | ribosomal protein |
| *Quercus rubra*[c] | FS_C8183 | $(AGC)_6$ | - | TATTCAACCACAGCTGCCTG | ACAGCTGCCTCTGTGGATCT | 200-213 | auxin response factor |

[a] gSSR developed in Sullivan et al. (2013); [b] Müller and Gailing (2018) and [c] Burger et al (2018), derived from a *Quercus rubra* EST-library (https://www.hardwoodgenomics.org/Transcriptome-assembly/1963023?tripal_pane=group_description_download). All other markers are EST-SSR, which were developed in Durand et al. (2010). LG denotes the linkage group on which the EST-SSR is located in *Q. robur* (Durand et al. 2010).

Genomic DNA from each of the 689 individual tree samples was amplified with six different multiplexes in a 13 µl PCR mix. The PCR mix of multiplex 1 (2P24, 3A05, 3D15) and multiplex 2 (FIR013, FIR028, FIR035) consisted of 1.5 µl reaction buffer (containing 0.8 M Tris-HCl and 0.2 M $(NH_4)_2SO_4$), 1.5 µl $MgCl_2$ (25 mM), 1 µl dNTPs (2.5 mM of each dNTP), 0.2 µl HOTFIREPol *Taq* polymerase (Solis BioDyne, Estonia) (5 units/µl), 5.8 µl $H_2O$, 0.5 µl of each forward primer (5 picomol/µl), 0.5 µl of each reverse primer (5 picomol/µl) and 1 µl DNA (ca. 0.6 ng/µl). The PCR mix of multiplex 3 (PIE125, GOT040, VIT023, VIT107) consisted of 6.5 µl Multiplex *Taq* PCR Master Mix Kit (QIAGEN, Germantown, Maryland, USA, providing a final concentration of 3 mM $MgCl_2$), 2.8 µl $H_2O$, 0.4 µl of each forward and reverse primer PIE125 (5 picomol/µl), 0.7 µl of each forward and reverse primer VIT107 (5 picomol/µl), 0.25µl of each forward and reverse primer VIT023 (5 picomol/µl), 0.5 µl of each forward and reverse primer GOT040 (5 picomol/µl) and 1 µl DNA (ca. 0.6 ng/µl). For multiplex 4 (PIE102, FIR104, PIE267), the PCR mix consisted of 1.5 µl reaction buffer (containing 0.8 M Tris-HCl and 0.2 M $(NH_4)_2SO_4$), 1.5 µl $MgCl_2$ (25 mM), 1 µl dNTPs (2.5 mM of each dNTP), 0.2 µl HOTFIREPol *Taq* polymerase (Solis BioDyne, Estonia) (5 units/µl), 6.2 µl $H_2O$, each 0.5 µl forward and reverse primer PIE102 (5 picomol/µl) and FIR104 (5 picomol/µl), 0.3 µl of each forward and reverse primer PIE267 (5 picomol/µl) and 1 µl DNA (ca. 0.6 ng/µl). For PCR amplifications of multiplex 5 (FS_C032, FS_C2660, FS_C2791, FS_C8183) and 6 (Qr0057, Qr1423, FS_C2361) a cost-effective tailed-primer approach was used (Schuelke 2000; Kubisiak et al. 2009) consisting of 1.5 µl reaction buffer (containing 0.8 M Tris-HCl and 0.2 M $(NH_4)_2SO_4$), 1.5 µl $MgCl_2$ (25 mM), 1 µl dNTPs (2.5 mM of each dNTP), 0.2 µl HOTFIREPol Taq polymerase (Solis BioDyne, Estonia) (5 units/µl), 5.5 µl $H_2O$, 0.2 µl M13 (5'-CACGACGTTGTAAAACGAC-3´) (Kubisiak et al. 2009) tailed forward primer (5 picomole/µl), 0.5 µl PIG-tailed reverse primer (5'-GTTTCTT-3') (5 picomole/µl) (Brownstein et al. 1996; Schuelke 2000; Kubisiak et al. 2009), 1 µl M13 (6-FAM/HEX) primer (5 picomole/µl), 5µl $H_2O$ (5.7 µl $H_2O$ for multiplex 6) and 1 µl DNA (ca. 0.6 ng/µl).

All PCR reactions were performed in a Biometra Thermal Cycler (MJ Research PTC 200, Analytik Jena, Germany) with a touchdown program. The PCR protocol for each marker was as follows: 15 min initial denaturation at 95°C followed by 10 touchdown cycles at 94°C for 1 min, 1 min at 60°C (decreasing 1 °C each cycle) and 1 min at 72°C, followed by 25 cycles at 94°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 1 min, and a final extension step at 72°C for 20 min. The PCR amplification was tested on 1.5% agarose gels in 1x TAE buffer. Amplification products were resolved on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, USA) using the GeneScan™ Rox-500 and Liz-500 (only for multiplex 3) size markers. For the fragment length analysis, multiplexes 5 and 6 were run together. Scoring of alleles was conducted using GeneMapper® version 4.1 (Applied Biosystems, Foster City, USA).

### 3.2.3 Statistical data analysis

Genetic variation in populations was calculated as the number of alleles per locus ($N_a$), allelic richness ($A_r$) observed heterozygosity ($H_o$) and expected heterozygosity ($H_e$) in GenAlEx version 6.51b2 (Peakall and Smouse 2006, 2012; Smouse et al. 2017). Inbreeding coefficients ($F_{IS}$) and their significance were determined using the Fstat version 2.9.4 software (Goudet 2003). Significant deviations from zero were determined after Bonferroni correction ($\alpha$=0.05, p< 0.00007) implemented in the software Fstat (Goudet 2003) to compensate for type I errors. Allelic richness ($A_r$) was also calculated using Fstat. In addition, linkage disequilibrium (LD) was calculated for each pair of loci in the 37 populations using Genepop version 4.7.2 (Rousset 2008) based on the following settings: dememorization 10000, batches 100 and iterations per batch 5000.

The software BOTTLENECK version 1.2.02 (Piry et al. 1999) was used to detect signatures of recent genetic bottlenecks. Therefore, we performed a Wilcoxon signed-rank test (one-tailed) for heterozygosity excess for 'the infinite alleles model' (IAM) and 'the stepwise mutation model' (SMM) and a mode-shift analysis to test for a distortion in the allele frequency distribution.

To measure the genetic variation among populations, an analysis of molecular variance (AMOVA) was performed with GenAlEx using 9999 permutations. The genetic differentiation among populations was also calculated as the fixation index $F_{ST}$, $G_{ST}$ and Hedrick's standardized $G_{ST}$ ($G'_{ST}$(Hed)) for individual markers and across all markers in GenAlEx. Besides, a principal coordinate analysis (PCoA) was performed in GenAlEx based on the genetic distance implemented in GenAlEx between populations in order to find and plot the major patterns within this dataset (Peakall and Smouse 2006, 2012).

The Windows®-based software MicroChecker version 2.2.3 (van Oosterhout et al. 2004) was used to identify genotyping errors due to non-amplified alleles (null alleles) which can lead to overestimates of the inbreeding coefficient ($F_{IS}$). Arlequin version 3.5.2.2 (Excoffier and Lischer 2010) was run with 50,000 simulations of 100 demes per group with the infinite island model based on $F_{ST}$ in order to detect outliers which deviate significantly from the variation and differentiation expected under neutrality.

The calculation of population structure was performed using STRUCTURE version 2.3.4 (Pritchard et al. 2000) to identify possible subpopulations based on the microsatellite dataset. Here, we tested 2-40 possible populations with ten runs per each K. The admixture model and correlated allele frequencies were selected initially, where a burn-in period of 50,000, Markov-Chain-Monte-Carlo (MCMC) repetitions of 100,000 and the LOCPRIOR model were used. However, we used additionally the default setting in STRUCTURE (admixed model without LOCPRIOR) to identify population structure solely based on genetic information. We also used the "no admixture" model in STRUCTURE to test if there is a clearer differentiation between the native and the Slavonian populations. The online program STRUCTURE HARVESTER v. 0.6.94 (Earl and von Holdt 2012) was used to determine the 'Best K' from

the logarithmic results and the *ΔK* method (Evanno et al. 2005). In addition, the CLUMPAK software (Cluster Markov Packager Across K) was used to post-process the results of the model-based population structure analysis (Kopelman et al. 2015).

A small proportion of indigenous haplotypes are also found in populations characterized as Slavonian oaks and vice versa. Therefore, additional STRUCTURE and principal component analysis were performed, for which individuals with the indigenous haplotypes (HP1, 4 and 10) in Slavonian populations (such as Tomberg 10B2, Kanitz 77C, Estermann 116A1) and individuals with the Slavonian haplotypes (HP5 and 7-26) in indigenous oak populations (Gut Ulenburg 4C, Kottenforst 134A&C) were removed (see Table S.1).

The software GeneClass2 was used to assign three populations (Tomberg 10B2, Freiherr v. der Leyen 17C and Kanitz 32H/39A) with unknown origin (see Table 1) to reference populations (here: *Q. robur* and *Q. robur* subsp*. slavonica* stands) based on multilocus data (Piry et al. 2004). These population assignments are modeled on Nei's standard distance (Nei 1972), Goldstein's distance (Goldstein et al. 1995) and using the Bayesian method (Baudouin and Lebrun 2001) taking the allele size of the SSRs into account (Piry et al. 2004). GeneClass was also used to perform self-assignment simulations among the individuals using the leave-one-out procedure.

## 3.3 Results

### 3.3.1 Genetic variation within populations

Inbreeding coefficients across all markers were not significantly different from zero in any population. The mean expected heterozygosity ranged from 0.469 in Graf Merveldt 3A to 0.566 in Plettenberg 106G and the mean observed heterozygosity ranged from 0.43 in Blix Flur 2/155 to 0.552 in Kottenforst 70D (Kottenforst 154B is not representative due to the low sample size of 4 samples). A summary of the genetic parameters across all loci is given in Table S. 3. The observed and expected heterozygosity and inbreeding coefficient were lower (not significant) in the group of indigenous oaks (mean $H_o$: 0.484, $H_e$:0.526, $F_{IS}$: 0.082) compared to Slavonian oaks (mean $H_o$: 0.490, $H_e$:0.540, $F_{IS}$: 0.093).

The mean expected heterozygosity per locus ranged from 0.117 at locus 3A05 to 0.863 at locus VIT107 and the mean observed heterozygosity per locus ranged from 0.109 at locus 2A05 to 0.837 at locus GOT040 (Table S. 7).

The genetic differentiation ($F_{ST}$) between the 37 populations was relatively low at most loci ranging from 0.025 for VIT023 to 0.062 for PIE125. Overall, the mean differentiation among all populations was 0.041 ($G_{ST}$=0.011, $G'_{ST}$(Hed)=0.027) (Online-Ressource 1). The mean pairwise $F_{ST}$ between indigenous oaks is 0.014 ($G_{ST}$=0.003, $G'_{ST}$(Hed)=0.008), between Slavonian oaks 0.018 ($G_{ST}$=0.003, $G'_{ST}$(Hed)=0.010) and between Slavonian and indigenous oaks 0.023 ($G_{ST}$=0.010, $G'_{ST}$(Hed)=0.034). Despite of the low

mean pairwise $F_{ST}$ value (0.024) between Slavonian and local oaks, these two groups were distinguished in the PCoA (Fig. 3).

### 3.3.2 Genetic variation among populations

The outlier tests with Arlequin showed that there are no markers with signatures of selection among populations and between groups (Slavonian vs. common oak) (Fig. S. 3).

There were no loci in LD in any population. Null alleles were detected in 31 populations at least at one of the markers 3D15, FIR028 (highest null allele frequency=0.3015 in Kanitz 19A), FIR035, PIE125, VIT023, VIT107, FIR104, PIE102, PIE267, Qr0057, Qr0332 (highest null allele frequency=0.3079 in Kanitz 77), Qr1423 and FS_C8183 (Table S. 2). However, in all populations except Graf Merveldt 3A, Kottenforst 85B, Gut Ulenburg 4C, Königsforst 127c, Kottenforst 70D and Kottenforst 154B null alleles only occurred with a frequency between 0.51 % and 5.51 % over all loci (Table S. 2). Null alleles across all populations per locus ranged from 0.005 at loci FS_C1423 and FS_C8183 to 0.064 at locus FIR035 (Table S. 7). Furthermore, comparison of null alleles between marker types showed that only one of the three gSSRs (3D15) showed null alleles with a low frequency (0.045), the null allele frequencies of EST-SSRs developed for *Q. robur* ranged from 0.006 at locus VIT023 to 0.126 at locus FIR028 and the null allele frequencies of EST SSRs developed for *Q. rubra* ranged from 0.005 at loci FS_C8183 and FS_C1423 to 0.028 at locus Qr0057 (Table S. 7). Across all loci, EST-SSRs developed for *Q. robur* have the highest null allele frequency (0.029), followed by gSSRs with 0.015 and EST-SSRs developed for *Q. rubra* (0.007).

The results of the program BOTTLENECK show that the allele frequencies of all populations except Kottenforst 154B followed a normal L-shaped distribution (Table S. 8). The AMOVA showed that 99 % of the molecular variance was within populations (9 % between individuals and 90 % within individuals) and only 1 % among populations.

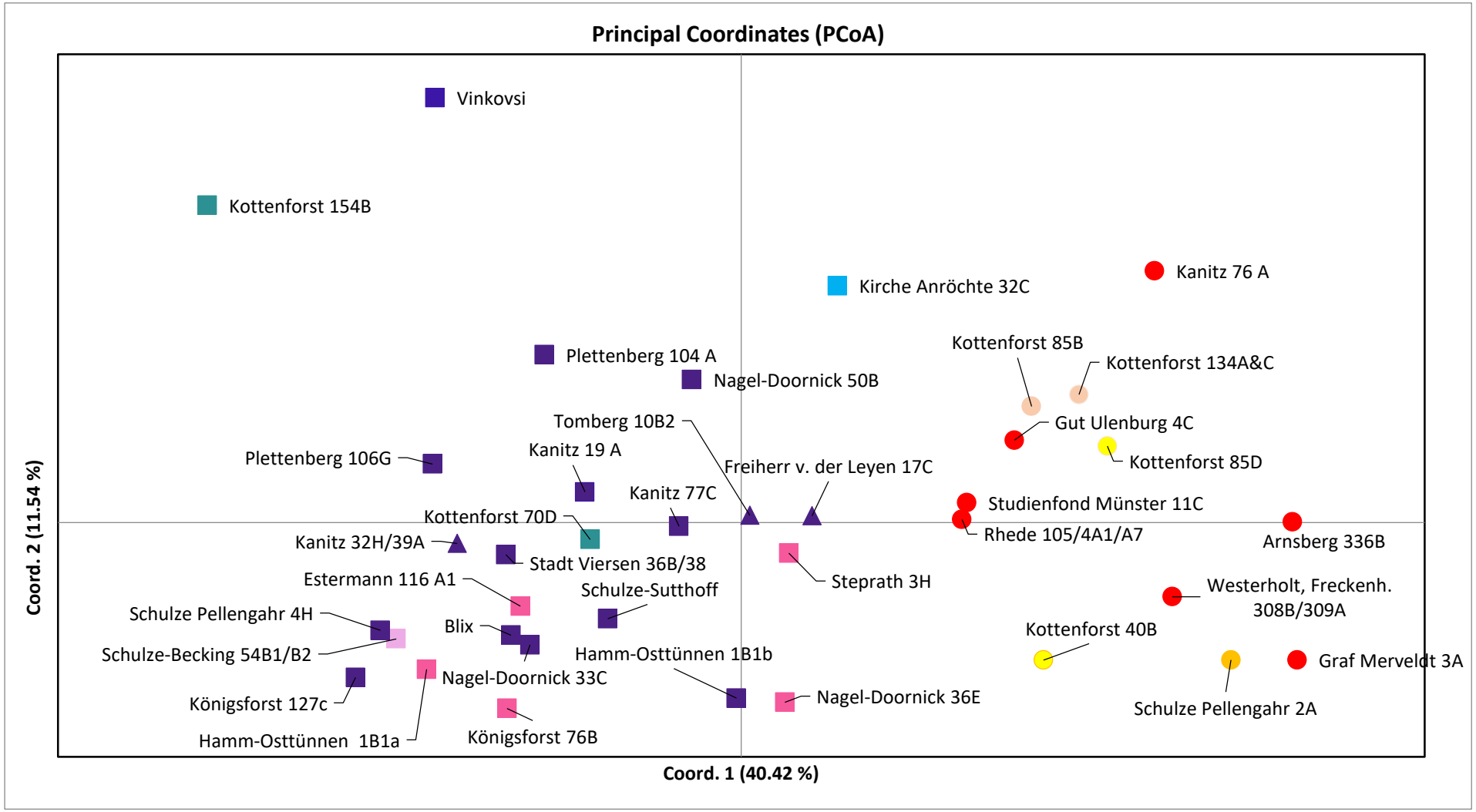


**Figure 3** Principal coordinate analysis (PCoA) based on nSSRs for all populations. HP=haplotype. Symbols square=phenologically characterized as Slavonian oak, circle=common oak, triangle=not clearly defined. The different colours stand for the most common haplotypes (Petit et al. 2002): red=HP 1, yellow=HP 10, orange=HP 12, blue=HP7-26, purple=HP5, pink=HP 2. The colour light purple means that both haplotype 2 and haplotype 5 (Schulze-Becking 54B1/B2) occur. The same applies to the colour turquoise, where both haplotype 5 and haplotype 17 (Kottenforst 70D) occur. The colour light orange stands for the occurrence of haplotypes 1, 4 and 10 (Kottenforst 134A&C, Kottenforst 85B).

### 3.3.3 Structure analysis of populations

Using the Evanno method and STRUCTURE HARVESTER an optimal K (ΔK)=2 was determined (Fig. S. 1, Table S. 2, Table S. 4). The diagram calculated by STRUCTURE using the admixed model and LOCPRIOR with the population assumption of K=2 is presented in Figure 5. The results of the STRUCTURE analysis for K=3 and K=4 are presented in the supplementary material (Fig. S. 6, 7). In addition, Figure 2 and Table S. 1 show the proportion of membership of each sampled population in the 2 clusters, but there is no specific cluster for each origin, since both clusters occur in both origins. A proportion of ancestry >0.5 or <0.5 in cluster 1 is characteristic for indigenous oak and Slavonian stands, respectively. The results of the PCoA (Fig. 3) show that the stands of common oaks and Slavonian oaks are represented by two clusters which are weakly differentiated. In addition, Figure 4 shows a clearer distinction between Slavonian and local populations, as individuals with local haplotypes in Slavonian populations and individuals with Slavonian haplotypes in local populations (Table S. 1) were excluded (see chapter 'plant material'). However, after the exclusion of these individuals, the results in STRUCTURE were similar (Fig. S. 4). The results using the default setting in STRUCTURE without LOCPRIOR also show genetic differentiation of native and Slavonian populations, but less pronounced (Fig. S. 8, 9, 10). Besides, the results of the STRUCTURE "no admixture" model (K=2, 3 and 4) provided an even clearer distinction between native and Slavonian populations (Fig. S. 11, 12, 13). A further analysis in STRUCTURE shows that the Slavonian and native haplotypes are differentiated at nSSRs (Fig. S. 5).

The GeneClass analysis provided similar results as STRUCTURE and PCoA (see Table S. 1 and Table S. 5). According to the PCoA all three populations (Tomberg 10B2, Freiherr v. der Leyen 17C and Kanitz 32H/39A) cluster with Slavonian oak stands. However, using STRUCTURE Tomberg 10B2 and Freiherr v. der Leyen 17C show an assignment of 50% per cluster, only Kanitz 32H/39A belongs to Cluster 2 with more than 60% which is characteristic for Slavonian stands. Based on Nei's distance and the Bayesian approach, GeneClass assigns all three populations to Slavonian oak, whereas based on Goldstein's distance Tomberg is assigned to indigenous oak (see Table S. 5). The self-assignment test of GeneClass based on the Bayesian method showed that 83.5% of the individuals were assigned to the correct group (Slavonian or native oak) (Online-Ressource 2).

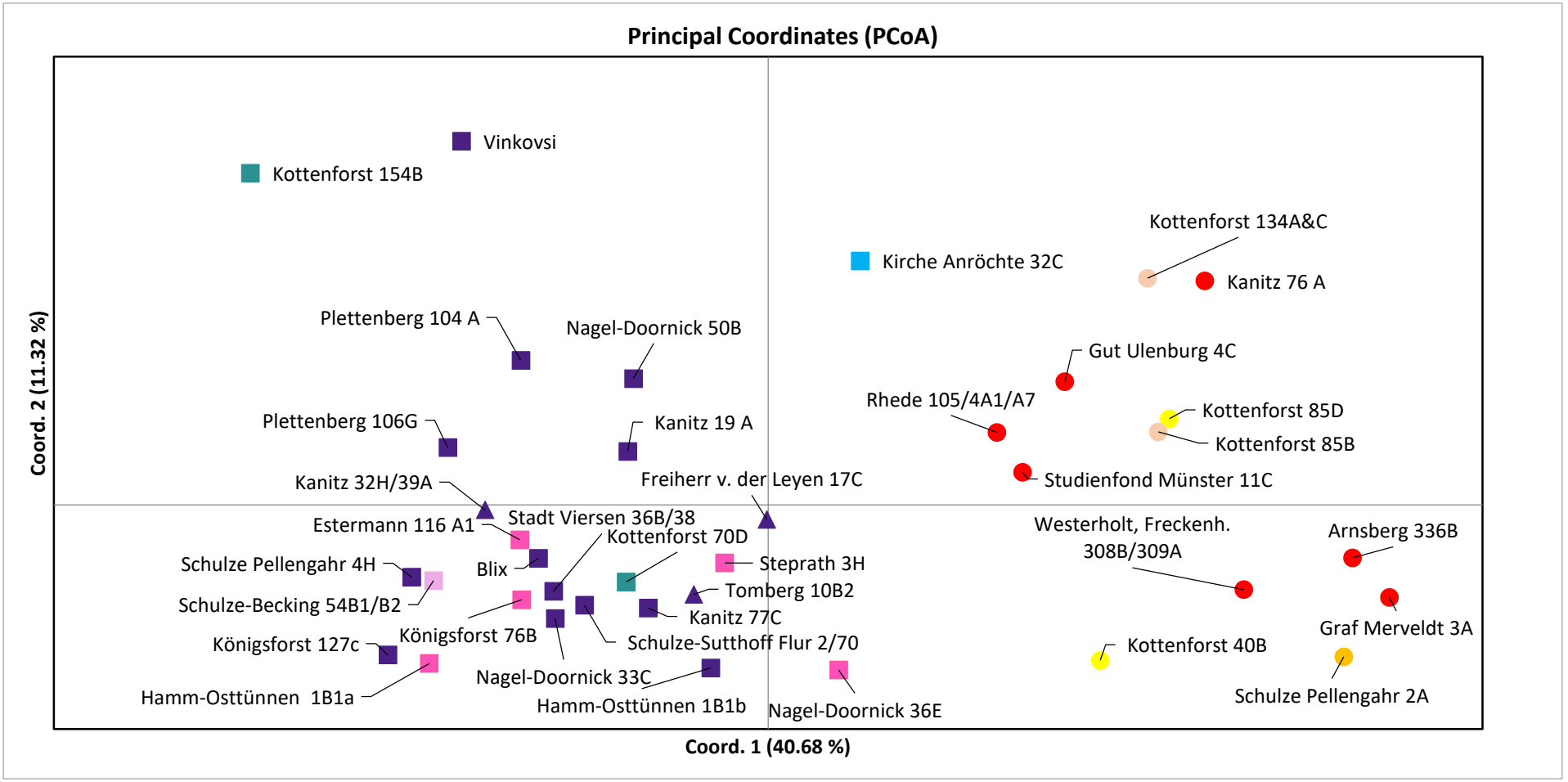


**Figure 4** Principal coordinate analysis (PCoA) based on nSSRs for all populations without Slavonian haplotypes in common oaks and common haplotypes in Slavonian oaks. HP=haplotype. Squares=phenologically characterized as Slavonian oak, circles=common oak, triangles=not clearly defined. The different colours stand for the most common haplotypes (Petit et al. 2002): red=HP 1, yellow=HP 10, orange=HP 12, blue=HP7-26, purple=HP5, pink=HP 2. The colour light purple means that both haplotype 2 and haplotype 5 (Schulze-Becking 54B1/B2) occur. The same applies to the colour turquoise, where both haplotype 5 and haplotype 17 (Kottenforst 70D) occur. The colour light orange stands for the occurrence of haplotypes 1, 4 and 10 (Kottenforst 134A&C, Kottenforst 85B).

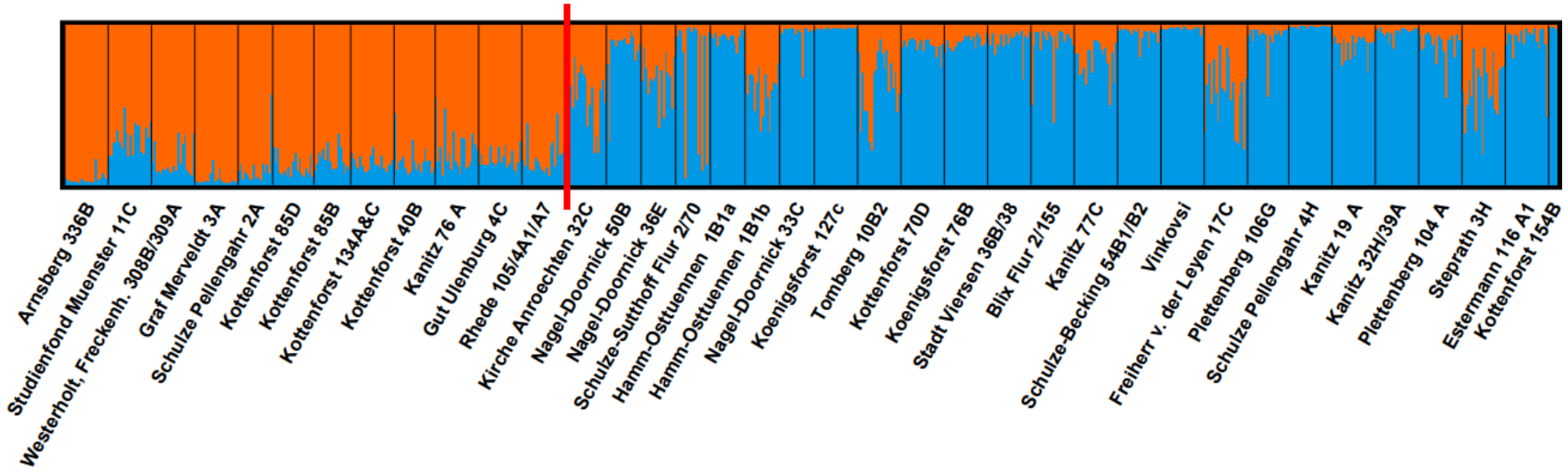


**Figure 5** STRUCTURE diagram with admixed model and LOCPRIOR for K=2. Blue=Slavonian oak, Orange=common oak. The red line divides the indigenous oaks (left) from the Slavonian oaks (right).

## 3.4 Discussion

The stands of oaks known as late-flushing oaks in North Rhine-Westphalia are well studied in terms of chloroplast DNA markers (Gailing et al. 2007a, b, 2009). To our knowledge, however, this is the first study on genetic differentiation between indigenous and Slavonian oak stands using nuclear microsatellites. The high transferability of EST-SSRs among species in *Quercus* and the availability of a large number of nSSRs and EST-SSRs (Steinkellner et al. 1997; Barreneche et al. 2004), made it possible to study 13 indigenous, 21 oak stands phenotypically described as Slavonian oak and 3 oak stands, for which it was not certain whether they are Slavonian oaks, based on a set of 20 nuclear microsatellite markers (Table S. 7 and Table 2).

### 3.4.1 Amount of genetic variation

Our dataset exhibits high levels of genetic diversity at the SSR loci examined (see Table S. 3) for all populations including the introduced Slavonian stands (e.g., $H_e$ ranged from 0.450 to 0.566). This relatively high level of genetic variation, as measured by basic statistics, was very similar across populations (see Table S.3) and is common for woody species (Hamrick et al. 1992). In addition, similar diversity values of the introduced plantations as compared to native stands, suggest that Slavonian stands were established with seed material that was sampled in Croatia from a representative number of trees per population.

The relatively high heterozygosity and allelic diversity allows for a good resolution of the underlying population genetic structures. Null alleles occurred with a frequency between 0.51% and 5.51% across all loci (Table S. 2). According to Oddou-Muratorio et al. (2009), however, null allele frequencies between 5% and 8% on average across loci are not expected to have effects on population genetic analyses. The markers selected for this study are also located on different linkage groups (see Table 2) and dispersed rather regularly across the genome (Durand et al. 2010). Also, the absence of linkage disequilibrium between marker pairs suggests that we have adapted a representative marker set.

Furthermore, the 10 EST-SSR markers developed for *Q. robur* (Durand et al. 2010) (see Table S. 3) revealed similar levels of genetic variation as in Bodénès et al. (2012) ($A_r$=4.20, $H_o$=0.51, $H_e$=0.74 and $A_r$=2.96, $H_o$=0.41, $H_e$=0.53). However, higher $N_a$, $H_e$ and $H_o$ values on average were revealed by Crăciunesc et al. (2011) ($N_a$=13.43, $H_o$=0.723 and $H_e$=0.769), Streiff et al (1998) ($H_o$=0.81 and $H_e$=0.87) and Neophytou et al. (2010) ($H_o$=0.741 and $H_e$=0.814) at potentially neutral gSSRs which are generally more variable than EST-SSRs (Ellis and Burke 2007; Buonaccorsi et al. 2012; Harmon et al. 2017). The total variation of all oaks in the present study ($N_a$=10.55, $A_r$=10.54, $H_o$=0.488, $H_e$=0.539) corresponds to the total variation of the Slavonian oaks ($N_a$=9.85, $A_r$=8.689, $H_o$=0.490, $H_e$=0.540) (see Table S.3). In addition, both the mean within stand variation and total variation are similar in Slavonian and indigenous stands (see Table S.3). Since neither the expected heterozygosity nor the number of alleles

per locus and allelic richness, both for variation within stands and total variation, is significantly lower in the Slavonian populations (Table S. 3), there is no evidence of losses in allelic richness and thus no evidence of a bottleneck effect (Nei et al. 1975). In addition, no evidence of a recent genetic bottleneck was identified in the microsatellite data using the infinite alleles and stepwise mutation models (Table S. 8). The fact that Kottenforst 154B shows a shifted mode in the allele frequency distribution is probably due to the low sample size of only 4 individuals. Therefore, there is no indication that the seed transfer of Slavonian oaks to Germany through humans reduced the variation within stands. Furthermore, the mean variation across all populations is almost equal to the total variation (see Table S. 3) and also the pairwise $F_{ST}$ values between Slavonian and indigenous oaks are low (0.024) (see Table S. 7), providing no evidence for fixation or genetic drift. A hierarchical division of the total genetic diversity for the two taxa also reveals that most of the variation is within populations (99 %), of which a comparatively high amount is within individuals (90 %). Differentiation among populations is low (1 %). However, pedunculate oaks almost always show low genetic differentiation between populations and high variation within populations (Zanetto et al. 1994; Gömöry et al. 2001; Mariette et al. 2002; Neophytou 2015).

### 3.4.2 Differentiation of stands

The differentiation between Slavonian and indigenous pedunculate oaks with nuclear microsatellite markers worked well at the population level, but less well at the individual level. This can be seen on the one hand in the PCoA based on populations that classifies the populations into two groups (Fig. 4). Only the two populations Kottenforst 154B and Vinkovsi are slightly separated within the Slavonian group. For population Kottenforst 154B this is probably due to the small sample size of only 4 individuals. For population Vinkovsi, the larger differences could be due to the fact that seeds were sampled that came directly from Vinkovsi in Croatia (Gailing et al. 2007b) and not from old growth first generation forest stands (Gailing et al. 2007a, b, 2009).

On the other hand, the differentiation between taxa is also shown in STRUCTURE results based on individuals (Pritchard et al. 2000; Evanno et al. 2005; Kalinowski 2011), but the differentiation is not as pronounced as in the PCoA (see Fig. 5 and Fig. S. 4). It shows that the assignment of individuals to clusters as in STRUCTURE is not suitable for genetically slightly differentiated taxa. In addition to these two methods (PCoA and STRUCTURE) there are a few more methods for population structure analysis such as LAMP (Sankararaman et al. 2008; Paşaniuc et al. 2009; Baran et al. 2012), ADMIXTURE (Alexander et al. 2009; Alexander and Lange 2011; Zhou et al. 2011) and FRAPPE (Tang et al. 2005; Alexander et al. 2009). However, these programs also have some disadvantages like no explicit account for LD between markers (ADMIXTURE), applicability only for SNP data (LAMP) or the estimation can be

slightly inaccurate (FRAPPE), hence STRUCTURE and PCoA were chosen as the most common methods for the analysis (Porras-Hurtado et al. 2013).

In addition to these nuclear marker results, the inclusion of cpDNA haplotypes from Gailing et al. (2007a, b, 2009) shows that Slavonian oak stands are not always pure stands (see Table S.1). After the exclusion of indigenous haplotypes in Slavonian populations, the separation between the two groups detected by the PCoA was more pronounced (Fig. 4). A possible reason for the occurrence of indigenous haplotypes in stands established with Slavonian seeds is that indigenous seeds may have been used for replanting in these stands. On the other hand, an additional natural regeneration of the common oak by the Eurasian jay (*Garrulus glandarius*) cannot be excluded. According to Hafer and Bauer (1993) this bird can transport acorns over 5-8 km distances. For example, in nearby stands such as Kanitz 77C (90 % HP5 and 10 % HP1) and Kanitz 76A (100 % HP1) (Fig. 1: population number 10 and 26; Fig. 2) transport of seeds could be a reason for the occurrence of haplotype 1 in the Slavonian population Kanitz 77C.

Furthermore, there were three populations, Kanitz 32H/39A, Tomberg 10B2 and Freiherr v. d. Leyen 17C (Table 1, Table S. 1), which show predominantly haplotype 5, however, the Slavonian origin was unclear. Due to the phylogeographic variation patterns of the cpDNA haplotypes, cpDNA markers are particularly useful to infer the origin of oak trees (Petit et al. 2002; Gailing et al. 2003; Finkeldey et al. 2010; Finkeldey and Hattemer 2010). However, the exact geographic origin of populations with haplotype 5 is unclear, as it occurs naturally in Germany and Croatia. In addition to haplotype 5, Tomberg 10B2 and Freiherr v. d. Leyen 17C reveal a relatively high frequency (15 % and 20 %) of non-Slavonian haplotypes 1 and 10 (Table S. 1) suggesting a mixture of reproductive material or later replanting.

GeneClass was used to assign these three populations to one of the two origins indigenous or Slavonian oak (Piry et al. 2004). The population Kanitz 32H/39A can be assigned to a population of Slavonian origin, since all three methods (Nei's distance with 74 %, Goldstein's distance with 69 % and the Bayesian method with 100 %) assign this population to Slavonian oak. In addition, Freiherr v. d. Leyen 17 C can also be assigned to Slavonian oak, according to Goldstein's distance with 67 % and the Bayesian method with 99.9 %, but regarding Nei's distance only with 52 % (see Table S. 5). The assignment of these two populations also correspond to the STRUCTURE results (Freiherr v. d. Leyen 17C with 51 % and Kanitz 32H/39A with 64 % and those of the PCoA (see Table S.1)). Population Tomberg 10B2, however, cannot be clearly assigned, because according to STRUCTURE it reveals 50 % ancestry in each cluster, according to PCoA it is rather assigned to Slavonian oak (see Fig. 3, 4), according to GeneClass based on Nei's distance (55 %) and based on the Bayesian method (100 %) to Slavonian oak and based on Goldstein's distance (51 %) again to indigenous oak (see Table S. 1 and Table S.5). According to the results of the cpDNA haplotypes, the stands Tomberg 10B2 and Freiherr v.

der Leyen 17C show a low amount of indigenous haplotypes (HP1, HP10) compared to Kanitz 32H/39A consisting only of Slavonian haplotypes (see Table S.1). The occurrence of indigenous haplotypes in both of these stands might lead to the slightly lower population assignment to Slavonian oak (see Table S.5). After exclusion of the indigenous haplotypes HP1 and HP10 in Tomberg 10B2 and Freiherr v. der Leyen 17C GeneClass assigns the two stands as Slavonian oaks based on Nei's standard distance (Tomberg 10B2: 59 %, Leyen 17C: 57 %), Goldstein's distance (Tomberg 10B2: 58 %, Leyen 17C: 78 %) and also based on Bayesian analysis (Tomberg 10B2: 100 %, Leyen 17C: 100 %) (see Table S.6).

In conclusion, nuclear markers can be used to validate the geographic origin of populations with haplotypes, which occur both in Germany and in Croatia. The usefulness of nuclear markers to differentiate between individuals and populations of German and Slavonian origin was validated for individual trees and stands with haplotype 2, which does not occur naturally in Germany. A combination of the results of both marker types (cpDNA marker and nuclear SSRs) is necessary to differentiate between taxa that differ only slightly from each other and to identify admixture.

### 3.4.3 Practical relevance

In the course of climate change and the associated forest restructuring towards stable mixed stands, the Slavonian oak is becoming increasingly important. On the one hand, forest owners are showing interest in planting Slavonian oaks because of their high growth rate, very good quality characteristics, late bud burst and possibly better adaptation to climate change (Schirmer 2017). On the other hand, the Slavonian oak is also of great interest to many tree nurseries, as suitable oak seed is needed due to the decline in oak seedling harvest years and the desired forest restructuring (Schirmer 2017). For practical purposes, seed material with known geographic origin is needed for forest management geared towards the sustainable use of genetic resources ensuring the long-term adaptability of tree species (Geburek and Schüler 2012). Accordingly, the forest owner needs certainty about the origin of the planting material through certification of the origin of reproductive material. For this purpose, the marker set used in this study is suitable to identify mixed stands, to certify the origin of the reproductive material and to differentiate between Slavonian and native oak stands. Wypukol et al. (2008) also successfully tested beech (*Fagus sylvatica*) for varietal purity and identity using nuclear markers. Neophytou (2012) for instance used nuclear microsatellites to differentiate between oak species *Q. robur* and *Q. petraea*.

## 3.5 Summary and Outlook

The nuclear marker set tested in this study is useful for further studies to identify Slavonian stands which are already established in Germany (e.g. stands of late flushing oak Burg-Eltz in Rhineland-Palatinate) in order to investigate whether the Slavonian oak is an alternative variety with regard to

climate change. The low differentiation at genetic markers but phenotypic and phenological differences suggest different local adaptations of both taxa. Both taxa are likely differentiated at a few genomic regions which are associated with these differences. Outlier screens and QTL mapping will be used in future studies to identify these regions. Furthermore, the low genetic differences between the two oak taxa suggest that the Slavonian oak is not a separate subspecies of the common oak, but rather a variety adapted to the local microclimatic conditions in the region of origin. In general, sufficient reference samples from all Slavonian oaks in Croatia and Germany would be important to better delineate the origin of individual trees and stands. In addition, the natural rejuvenation of nearby indigenous and Slavonian oaks must be analyzed in order to estimate the amount of gene flow between the two taxa.

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Kapitel 3

# Supplementary Material

**Table S. 1** Proportion of membership of each pre-defined population. STRUCTURE Cluster 1 is characteristic for common oak populations and STRUCTURE cluster 2 for Slavonian oak populations. Also relative frequencies (rel. Freq.) of occurring haplotypes (HP) in each population are shown.

| population | Cluster 1 | Cluster 2 | HP[1)] | rel. Freq. |
|---|---|---|---|---|
| Graf Merveldt 3A[a] | 0.961 | 0.039 | 1 | 0.95 |
| | | | 12 | 0.05 |
| Arnsberg 336B[a] | 0.959 | 0.041 | 1 | 1 |
| Schulze Pellengahr 2A[a] | 0.882 | 0.118 | 12 | 0.69 |
| | | | 1 | 0.31 |
| Kottenforst 85D[a] | 0.838 | 0.162 | 10 | 1 |
| Kottenforst 40B[a] | 0.834 | 0.166 | 10 | 1 |
| Westerholt, Freckenh. 308B/309A[a] | 0.842 | 0.158 | 1 | 1 |
| Gut Ulenburg 4C[a] | 0.825 | 0.175 | 1 | 0.85 |
| | | | 10 | 0.05 |
| | | | **7-26** | 0.05 |
| Kottenforst 85B[a] | 0.823 | 0.177 | 10 | 0.12 |
| | | | 4 | 0.24 |
| | | | 1 | 0.59 |
| Kottenforst 134A&C[a] | 0.855 | 0.145 | 10 | 0.35 |
| | | | **5** | 0,1 |
| | | | 1 | 0.25 |
| | | | 4 | 0.3 |
| Rhede 105/4A1/A7[a] | 0.815 | 0.185 | 1 | 0.85 |
| | | | 10 | 0.15 |
| Studienfond Münster 11C[a] | 0.686 | 0.314 | 1 | 1 |
| Kanitz 76 A[a] | 0.772 | 0.228 | 1 | 1 |
| Hamm-Osttünnen 1B1b | 0.403 | 0.597 | 5 | 0.5 |
| | 0.961 | 0.039 | 2 | 0.25 |
| | | | 17 | 0.125 |
| | 0.959 | 0.041 | **1** | 0.125 |

| population | Cluster 1 | Cluster 2 | HP | rel. Freq. |
|---|---|---|---|---|
| Tomberg 10B2[b] | 0.365 | 0.635 | **1** | 0.1 |
| | | | **10** | 0.05 |
| | | | 5 | 0.8 |
| | | | 7-26 | 0.05 |
| Nagel-Doornick 36E | 0.335 | 0.665 | 2 | 1 |
| Freiherr v. der Leyen 17C[b] | 0.420 | 0.580 | 5 | 0.75 |
| | | | 5, 7-26 | 0.05 |
| | | | **1** | 0.05 |
| | | | **10** | 0.15 |
| Kirche Anröchte 32C | 0.440 | 0.560 | 7-26 | 0.63 |
| | | | 2 | 0.26 |
| | | | 5 | 0.05 |
| Steprath 3H | 0.385 | 0.615 | 2 | 0.8 |
| | | | **10** | 0.1 |
| | | | **1** | 0.05 |
| | | | 7-26 | 0.05 |
| Schulze-Sutthoff Flur 2/70 | 0.256 | 0.744 | 5 | 0.95 |
| Kanitz 77C | 0.213 | 0.787 | 5 | 0.9 |
| | | | **1** | 0.1 |
| Kottenforst 70D | 0.120 | 0.880 | 17 | 0.5 |
| | | | 5 | 0.45 |
| | | | 7-26 | 0.05 |
| Nagel-Doornick 50B | 0.155 | 0.845 | **1** | 0.125 |
| | | | 5 | 0.875 |
| Kanitz 19 A | 0.130 | 0.870 | 5, 7-26 | 0.15 |
| | | | 5 | 0.85 |

| population | Cluster 1 | Cluster 2 | HP | rel. Freq. |
|---|---|---|---|---|
| Stadt Viersen 36B/38 | 0.091 | 0.909 | 5 | 0.9 |
| | | | **4** | 0.05 |
| Königsforst 76B | 0.107 | 0.893 | 2 | 0.95 |
| Blix Flur 2/155 | 0.121 | 0.879 | 5 | 1 |
| Estermann 116 A1 | 0.097 | 0.903 | 2 | 0.85 |
| | | | **1** | 0.05 |
| | | | 7-26 | 0.05 |
| | | | **10** | 0.05 |
| Plettenberg 104 A | 0.115 | 0.623 | 5 | 0.95 |
| | | | 1 | 0.05 |
| Hamm-Osttünnen 1B1a | 0.066 | 0.934 | 5 | 0.31 |
| | | | 2 | 0.56 |
| | | | 7-26 | 0.13 |
| Kanitz 32H/39A[b] | 0.043 | 0.885 | 5 | 1 |
| Nagel-Doornick 33C | 0.043 | 0.957 | 5 | 1 |
| Schulze-Becking 54B1/B2 | 0.056 | 0.944 | 5 | 0.65 |
| | | | 2 | 0.3 |
| | | | 17 | 0.05 |
| Königsforst 127c | 0.024 | 0.976 | 5 | 1 |
| Plettenberg 106G | 0.071 | 0.929 | 5b | 1 |
| Schulze Pellengahr 4H | 0.009 | 0.991 | 5b | 1 |
| Vinkovsi | 0.023 | 0.977 | 5 | 1 |
| Kottenforst 154B | 0.021 | 0.979 | 17 | 0.5 |
| | | | 5b | 0.5 |

[a] morphologically characterized as indigenous oak, [b] Slavonian origin unclear, no sign=morphologically characterized as Slavonian oak, marked in bold face=haplotypes removed for additional analyses.
[1)]HP1: Italy-Scandinavia line (lineage C), HP2: Croatia-Sicily line (lineage C), HP4: central Europe line (lineage A), HP5: Italy-Eastern Balkan-Germany line (lineage A), HP7-26: Croatia- Catalonia line (lineage A), HP10,: Western Europe-Portugal line (lineage B), HP11, 12: Western Europe line (lineage B), HP17: Italy-Balkan line (lineage E) (Petit et al. 2002).

**Table S. 2** Presence of null alleles.

| population | locus | Frequency of null allele | across Loci |
|---|---|---|---|
| Arnsberg 336B | 3D15 | 0.2251 | 3.89% |
| | FIR028 | 0.1719 | |
| | PIE125 | 0.1574 | |
| | VIT107 | 0.0863 | |
| | FIR104 | 0.137 | |
| Studienfond Münster 11C | FIR028 | 0.1539 | 0.77% |
| Freckenh. 308B/309A | FIR028 | 0.2604 | 1.76% |
| | VIT107 | 0.0909 | |
| Schulze Pellengahr 2A | FIR028 | 0.1756 | 2.24% |
| | VIT107 | 0.1075 | |
| | FIR104 | 0.1641 | |
| Kottenforst 85D | VIT107 | 0.1026 | 0.51% |
| Kottenforst 134A&C | 3D15 | 0.2027 | 3.32% |
| | FIR035 | 0.1272 | |
| | VIT107 | 0.1275 | |
| | Qr0332 | 0.2073 | |
| Kottenforst 40B | 3D15 | 0.2787 | 4.01% |
| | FIR035 | 0.2304 | |
| | VIT107 | 0.1107 | |
| | PIE102 | 0.1824 | |
| Kanitz 76 A | FIR028 | 0.2362 | 3.99% |
| | FIR035 | 0.2172 | |
| | VIT107 | 0.098 | |
| | Qr0057 | 0.2472 | |
| Rhede 105/4A1/A7 | FIR028 | 0,1274 | 1.33% |
| | FIR035 | 0.1378 | |
| Kirche Anröchte 32C | FIR028 | 0.1593 | 1.87% |
| | VIT023 | 0.2154 | |
| Nagel-Doornick 50B | FIR028 | 0.1917 | 2.11% |
| | PIE102 | 0.2301 | |
| Nagel-Doornick 36E | FIR028 | 0.1589 | 0.79% |
| Schulze-Sutthoff Flur 2/70 | 3D15 | 0.2305 | 2.03% |
| | FIR028 | 0.1763 | |
| Hamm-Osttünnen 1B1a | 3D15 | 0.1865 | 0.93% |
| Hamm-Osttünnen 1B1b | FIR028 | 0.1408 | 0.70% |
| Nagel-Doornick 33C | FS_C8183 | 0.1865 | 0.93% |
| Tomberg 10B2 | FIR028 | 0.1561 | 0.78% |
| Königsforst 76B | 3D15 | 0.2879 | 3.92% |
| | VIT107 | 0.1359 | |
| | PIE102 | 0.2642 | |
| | PIE267 | 0.0966 | |
| Stadt Viersen 36B/38 | FIR028 | 0.2079 | 2.20% |
| | FIR035 | 0.2322 | |
| Blix Flur 2/155 | FIR028 | 0.2432 | 3.01% |
| | FIR035 | 0.1546 | |
| | Qr0057 | 0.2035 | |
| Kanitz 77C | FIR028 | 0.1894 | 4.31% |
| | FIR035 | 0.2013 | |
| | PIE102 | 0.163 | |
| | Qr0057 | 0.3079 | |
| Schulze-Becking 54B1/B2 | FIR028 | 0.2693 | 4.11% |
| | VIT107 | 0.0756 | |
| | PIE102 | 0.213 | |
| | Qr0057 | 0.2639 | |
| Vinkovsi | FIR028 | 0.2738 | 2.86% |
| | FIR035 | 0.2977 | |
| Freiherr v. der Leyen 17C | FIR028 | 0.2254 | 5.51% |
| | FIR035 | 0.2912 | |
| | PIE125 | 0.1973 | |
| | PIE102 | 0.2092 | |
| | Qr0332 | 0.1792 | |
| Plettenberg 106G | FIR028 | 0.2688 | 1.99% |
| | PIE267 | 0.1293 | |
| Schulze Pellengahr 4H | 3D15 | 0.2382 | 3.41% |
| | FIR028 | 0.1439 | |
| | FIR035 | 0.1971 | |
| | VIT107 | 0.1025 | |
| Kanitz 19 A | FIR028 | 0.3015 | 2.12% |
| | FIR035 | 0.1232 | |
| Kanitz 32H/39A | FIR104 | 0.1753 | 0.88% |
| Plettenberg 104 A | FIR028 | 0.2464 | 1.95% |
| | PIE267 | 0.1433 | |
| Steprath 3H | FIR035 | 0.1616 | 0.81% |
| Estermann 116 A1 | FIR028 | 0.1797 | 2.45% |
| | VIT107 | 0.1366 | |
| | Qr1423 | 0.173 | |

**Table S. 3** Summary of genetic parameters: N=number of individuals, $N_a$=average number of alleles per gene locus, $A_r$=allelic richness, $H_o$=observed heterozygosity, $H_e$=expected heterozygosity, $F_{IS}$=inbreeding coefficient across all loci.

| Population | N | $N_a$ | $A_r$ | $H_o$ | $H_e$ | $F_{is}$ |
|---|---|---|---|---|---|---|
| Arnsberg 336B | 20 | 4.80 | 2.88 | 0.440 | 0.497 | 0.140 |
| Studienfond Münster 11C | 19.8 | 4.55 | 2.86 | 0.508 | 0.503 | 0.016 |
| Westerholt, Freckenh. 308B/309A | 20 | 5.15 | 2.94 | 0.448 | 0.500 | 0.13 |
| Graf Merveldt 3A | 20 | 4.15 | 2.72 | 0.468 | 0.469 | 0.029 |
| Schulze Pellengahr 2A | 16 | 4.65 | 2.86 | 0.466 | 0.479 | 0.06 |
| Kottenforst 85D | 19 | 4.70 | 3.00 | 0.497 | 0.518 | 0.066 |
| Kottenforst 85B | 17 | 4.95 | 3.07 | 0.497 | 0.517 | 0.068 |
| Kottenforst 134A&C | 20 | 5.40 | 3.18 | 0.510 | 0.546 | 0.091 |
| Kottenforst 40B | 19 | 4.95 | 2.97 | 0.482 | 0.515 | 0.092 |
| Kanitz 76 A | 20 | 5.10 | 3.12 | 0.495 | 0.536 | 0.102 |
| Gut Ulenburg 4C | 19.95 | 5.05 | 3.08 | 0.522 | 0.534 | 0.048 |
| Rhede 105/4A1/A7 | 20 | 5.25 | 3.00 | 0.478 | 0.498 | 0.066 |
| **mean across local oaks** | **19.23** | **4.89** | **2.97** | **0.48** | **0.51** | **0.08** |
| **Total variation local oaks** | **230.750** | **7.850** | **7.846** | **0.484** | **0.526** | **0.082** |
| Kirche Anröchte 32C | 19 | 5.35 | 3.20 | 0.497 | 0.538 | 0.102 |
| Nagel-Doornick 50B | 15.55 | 4.80 | 3.08 | 0.468 | 0.524 | 0.139 |
| Nagel-Doornick 36E | 15.95 | 5.15 | 3.15 | 0.536 | 0.529 | 0.018 |
| Schulze-Sutthoff Flur 2/70 | 15.9 | 5.05 | 3.05 | 0.487 | 0.518 | 0.092 |
| Hamm-Osttünnen 1B1a | 16 | 5.10 | 3.13 | 0.513 | 0.509 | 0.026 |
| Hamm-Osttünnen 1B1b | 16 | 4.95 | 3.12 | 0.550 | 0.521 | -0.023 |
| Nagel-Doornick 33C | 16 | 4.75 | 3.08 | 0.500 | 0.521 | 0.072 |
| Königsforst 127c | 20 | 510 | 3.11 | 0.543 | 0.527 | -0.004 |
| Tomberg 10B2 | 20 | 5.20 | 3.16 | 0.515 | 0.543 | 0.076 |
| Kottenforst 70D | 19.95 | 4.90 | 2.99 | 0.552 | 0.515 | -0.045 |
| Königsforst 76B | 20 | 5.40 | 3.12 | 0.485 | 0.515 | 0.083 |
| Stadt Viersen 36B/38 | 20 | 5.10 | 3.04 | 0.475 | 0.520 | 0.112 |
| Blix Flur 2/155 | 20 | 4.75 | 2.94 | 0.430 | 0.491 | 0.149 |
| Kanitz 77C | 20 | 5.20 | 3.07 | 0.478 | 0.524 | 0.114 |
| Schulze-Becking 54B1/B2 | 20 | 4.95 | 3.05 | 0.460 | 0.511 | 0.126 |
| Vinkovsi | 20 | 5.10 | 3.01 | 0.450 | 0.499 | 0.124 |
| Freiherr v. der Leyen 17C | 19.8 | 5.55 | 3.20 | 0.441 | 0.545 | 0.215 |
| Plettenberg 106G | 19 | 5.65 | 3.34 | 0.500 | 0.566 | 0.143 |
| Schulze Pellengahr 4H | 20 | 4.90 | 3.05 | 0.488 | 0.522 | 0.092 |
| Kanitz 19 A | 20 | 5.25 | 3.01 | 0.450 | 0.497 | 0.12 |
| Kanitz 32H/39A | 20 | 5.15 | 3.15 | 0.498 | 0.530 | 0.087 |
| Plettenberg 104 A | 20 | 5.15 | 3.13 | 0.483 | 0.520 | 0.097 |
| Steprath 3H | 20 | 5.35 | 3.22 | 0.515 | 0.549 | 0.087 |
| Estermann 116 A1 | 20 | 5.05 | 3.04 | 0.470 | 0.504 | 0.093 |
| Kottenforst 154B | 4 | 3 | 3 | 0.525 | 0.450 | -0.024 |
| **mean across Slavonian oaks** | **18.29** | **5.04** | **3.1** | **0.49** | **0.52** | **0.08** |
| **Total variation Slavonian oaks** | **457.150** | **9.850** | **8.689** | **0.49** | **0.54** | **0.093** |
| **Mean across all populations** | **18.592** | **4.989** | **3.057** | **0.49** | **0.516** | **0.081** |
| **Total variation** | **687.9** | **10.55** | **10.54** | **0.488** | **0.539** | **0.095** |

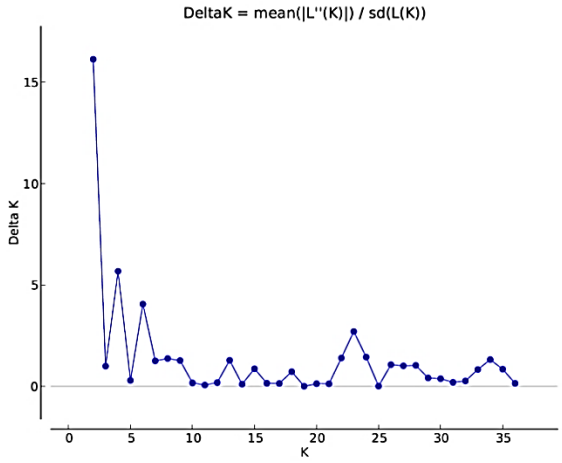


**Figure S. 1** STRUCTURE HARVESTER results to determine the most likely K value. The Evanno et al. (2005) method, which compares the ΔK between sequential K values. Function graph for determining the optimal K.

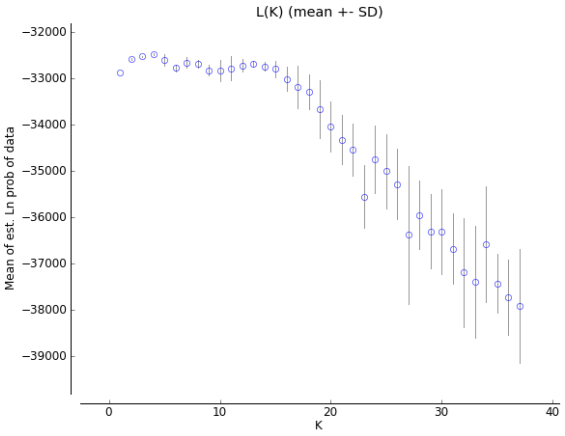


**Figure S. 2** Comparison of the mean of the estimate of the natural log of the probability of the data amongst K values, with the circle centered over the mean and the bar indicating the standard error.

**Table S. 4** Results of the Evanno method for the best K.

| K | Reps | Mean LnP(K) | Stdev LnP(K) | Ln'(K) | \|Ln''(K)\| | Delta K |
|---|---|---|---|---|---|---|
| 1 | 10 | -32.871.970.000 | 0.258414 | — | — | — |
| 2 | 10 | -32.590.900.000 | 13.741.098 | 281.070.000 | 221.630.000 | 16.128.988 |
| 3 | 10 | -32.531.460.000 | 13.962.665 | 59.440.000 | 14.030.000 | 1.004.823 |
| 4 | 10 | -32.486.050.000 | 30.945.337 | 45.410.000 | 175.820.000 | 5.681.631 |
| 5 | 10 | -32.616.460.000 | 127.085.939 | -130.410.000 | 38.400.000 | 0.302158 |
| 6 | 10 | -32.785.270.000 | 71.216.712 | -168.810.000 | 289.310.000 | 4.062.389 |
| 7 | 10 | -32.664.770.000 | 118.233.038 | 120.500.000 | 149.580.000 | 1.265.129 |
| 8 | 10 | -32.693.850.000 | 81.130.584 | -29.080.000 | 111.900.000 | 1.379.258 |
| 9 | 10 | -32.834.830.000 | 110.931.331 | -140.980.000 | 142.200.000 | 1.281.874 |
| 10 | 10 | -32.833.610.000 | 229.902.677 | 1.220.000 | 41.100.000 | 0.178771 |
| 11 | 10 | -32.791.290.000 | 258.088.204 | 42.320.000 | 18.480.000 | 0.071603 |
| 12 | 10 | -32.730.490.000 | 130.987.976 | 60.800.000 | 25.440.000 | 0.194216 |
| 13 | 10 | -32.695.130.000 | 67.487.432 | 35.360.000 | 87.130.000 | 1.291.055 |
| 14 | 10 | -32.746.900.000 | 85.184.127 | -51.770.000 | 9.720.000 | 0.114106 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 15 | 10 | -32.808.390.000 | 175.138.088 | -61.490.000 | 152.870.000 | 0.872854 |
| 16 | 10 | -33.022.750.000 | 263.286.119 | -214.360.000 | 42.280.000 | 0.160586 |
| 17 | 10 | -33.194.830.000 | 463.027.184 | -172.080.000 | 69.370.000 | 0.149818 |
| 18 | 10 | -33.297.540.000 | 372.562.607 | -102.710.000 | 271.420.000 | 0.728522 |
| 19 | 10 | -33.671.670.000 | 623.171.296 | -374.130.000 | 7.890.000 | 0.012661 |
| 20 | 10 | -34.037.910.000 | 542.315.629 | -366.240.000 | 75.090.000 | 0.138462 |
| 21 | 10 | -34.329.060.000 | 532.061.304 | -291.150.000 | 70.890.000 | 0.133237 |
| 22 | 10 | -34.549.320.000 | 559.610.648 | -220.260.000 | 786.150.000 | 1.404.816 |
| 23 | 10 | -35.555.730.000 | 667.862.696 | -1.006.410.000 | 1.811.440.000 | 2.712.294 |
| 24 | 10 | -34.750.700.000 | 734.134.291 | 805.030.000 | 1.065.000.000 | 1.450.688 |
| 25 | 10 | -35.010.670.000 | 794.579.264 | -259.970.000 | 14.100.000 | 0.017745 |
| 26 | 10 | -35.284.740.000 | 761.284.793 | -274.070.000 | 819.040.000 | 1.075.865 |
| 27 | 10 | -36.377.850.000 | 1.486.761.014 | -1.093.110.000 | 1.514.830.000 | 1.018.879 |
| 28 | 10 | -35.956.130.000 | 738.830.205 | 421.720.000 | 770.040.000 | 1.042.242 |
| 29 | 10 | -36.304.450.000 | 800.897.828 | -348.320.000 | 336.700.000 | 0.420403 |
| 30 | 10 | -36.316.070.000 | 918.785.983 | -11.620.000 | 352.130.000 | 0.383256 |
| 31 | 10 | -36.679.820.000 | 754.557.573 | -363.750.000 | 154.450.000 | 0.204689 |
| 32 | 10 | -37.198.020.000 | 1.168.226.874 | -518.200.000 | 320.550.000 | 0.274390 |
| 33 | 10 | -37.395.670.000 | 1.205.589.029 | -197.650.000 | 1.007.540.000 | 0.835724 |
| 34 | 10 | -36.585.780.000 | 1.245.067.507 | 809.890.000 | 1.654.170.000 | 1.328.579 |
| 35 | 10 | -37.430.060.000 | 632.366.345 | -844.280.000 | 539.490.000 | 0.853129 |
| 36 | 10 | -37.734.850.000 | 806.693.077 | -304.790.000 | 126.006.667 | 0.156201 |
| 37 | 6 | -37.913.633.333 | 1.230.478.760 | -178.783.333 | — | — |

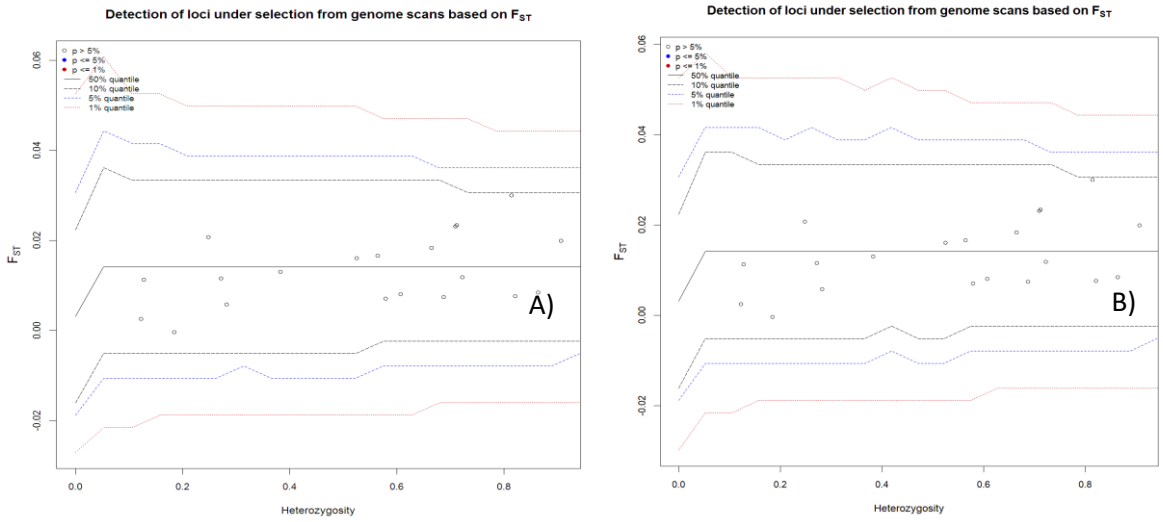


**Figure S. 3** A) Outlier analysis of 20 markers between indigenous and Slavonian stands and B) between all populations.

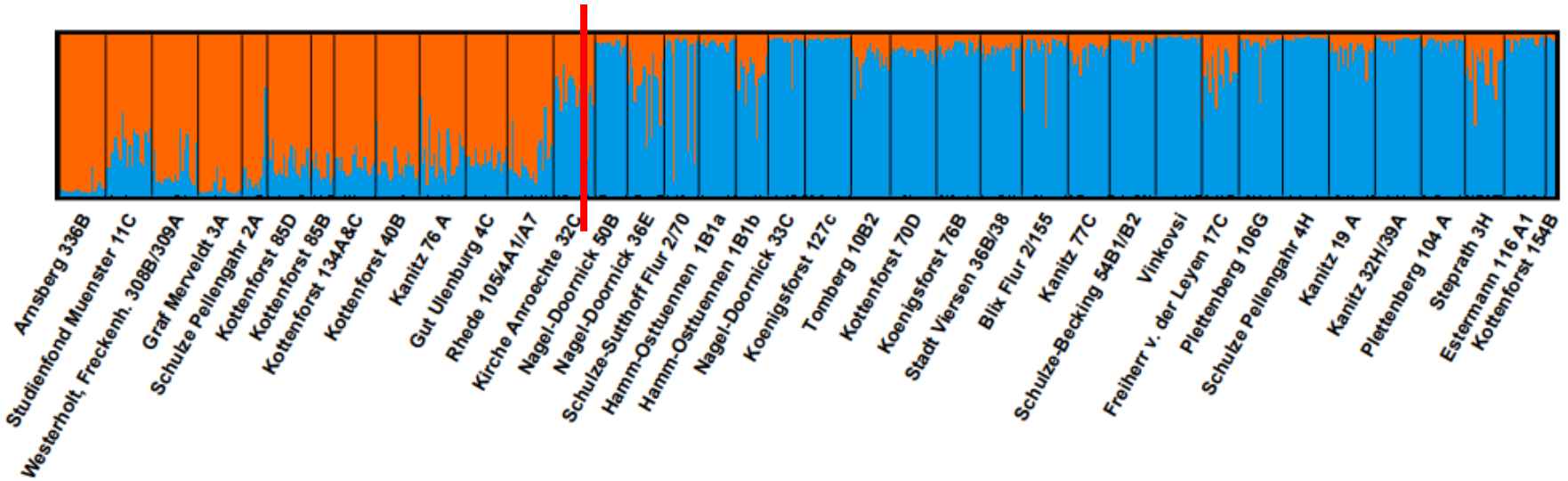


**Figure S. 4** STRUCTURE diagram with admixed model and LOCPRIOR for K=2. Blue=common oak, orange=Slavonian oak for all populations without Slavonian haplotypes in common oaks and common haplotypes in Slavonian oaks. The red line divides the indigenous oaks (left) from the Slavonian oaks (right).

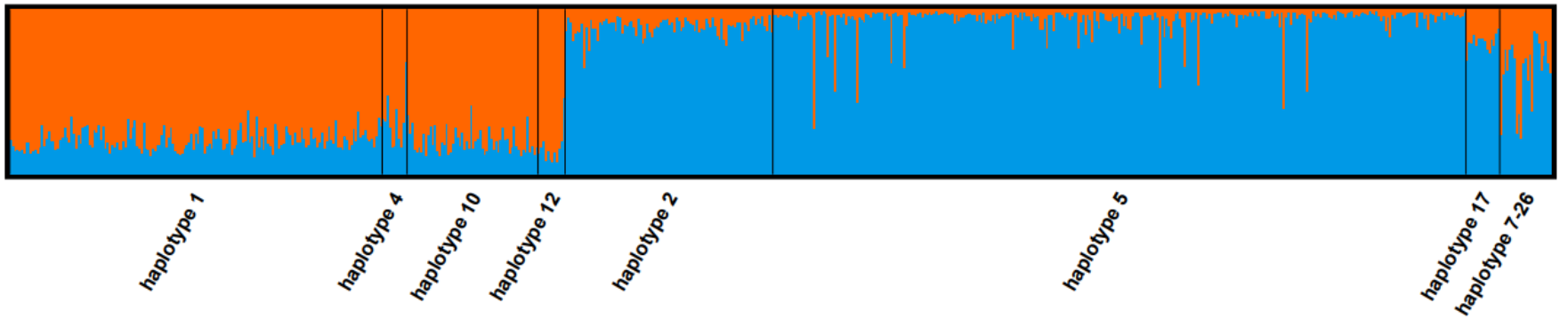


**Figure S. 5** Differentiation of haplotypes based on nSSRs (admixed model with LOCPRIOR). More orange=common haplotype, blue=slavonian haplotype.

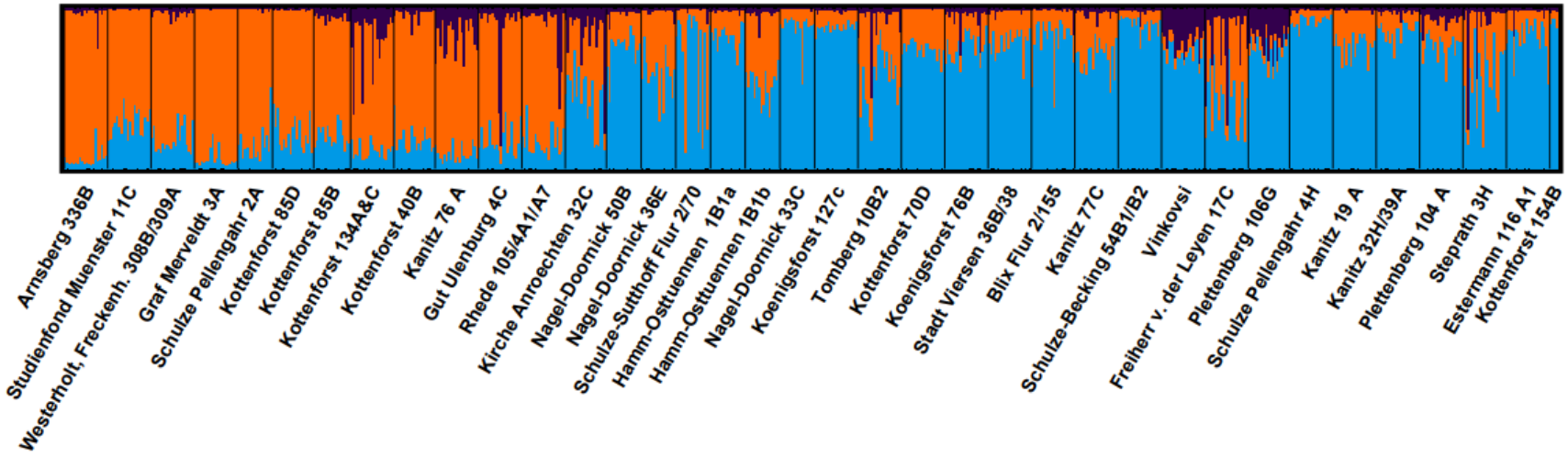


**Figure S. 6** STRUCTURE analysis with admixed model and LOCPRIOR for K=3 for all populations.

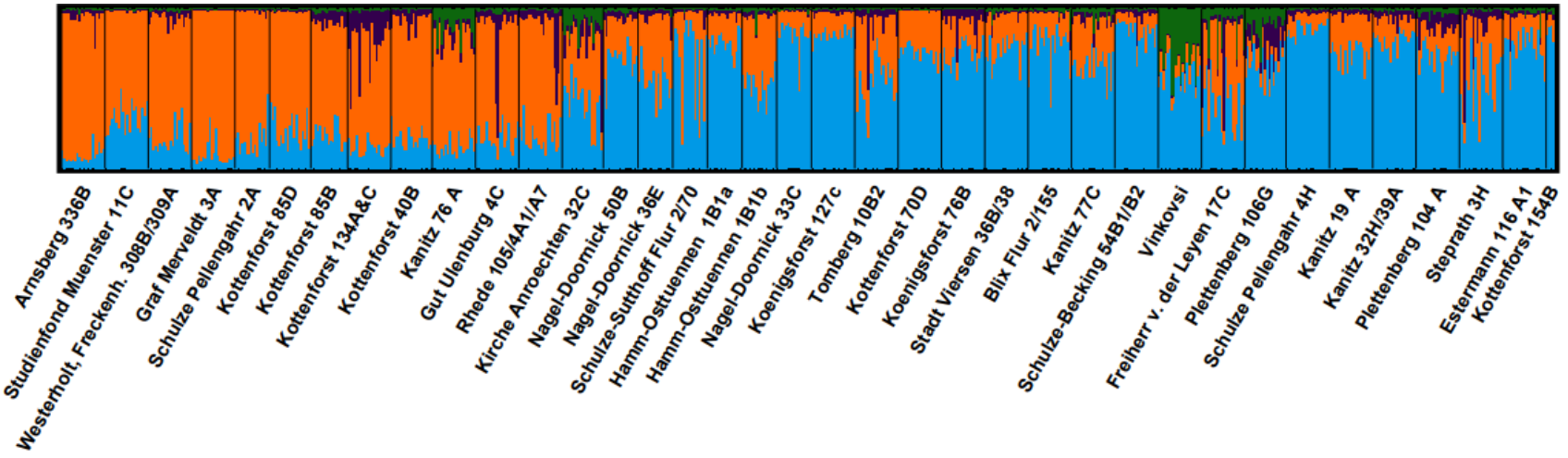


**Figure S. 7** STRUCTURE analysis with admixed model and LOCPRIOR for K=4 for all populations.

**Table S. 5** Results of the GeneClass analysis based on Nei's standard (Nei 1972) distance, distance of Goldstein et al. (1995) and Bayesian analysis (Baudouin and Lebrun 2001).

| **Nei's standard distance** | rank 1 | score % | rank 2 | score % | slavonian oak distance | indigenous oak distance |
|---|---|---|---|---|---|---|
| Tomberg 10B2 | slavonian oak | 54.853 | indigenous oak | 45.147 | 0.025 | 0.021 |
| Leyen 17C | slavonian oak | 52.553 | indigenous oak | 47.447 | 0.023 | 0.021 |
| Kanitz 32H/39A | slavonian oak | 73.995 | indigenous oak | 26.005 | 0.035 | 0.012 |

| **Goldstein** | rank 1 | score % | rank 2 | score % | slavonian oak distance | indigenous oak distance |
|---|---|---|---|---|---|---|
| Tomberg 10B2 | indigenous oak | 51.168 | slavonian oak | 48.832 | 0.641 | 0.672 |
| Leyen 17C | slavonian oak | 67.620 | indigenous oak | 32.380 | 0.766 | 0.367 |
| Kanitz 32H/39A | slavonian oak | 68.884 | indigenous oak | 31.116 | 1.205 | 0.545 |

| **Bayesian** | rank 1 | score % | rank 2 | score % |
|---|---|---|---|---|
| Tomberg 10B2 | slavonian oak | 100.000 | indigenous oak | 0.000 |
| Leyen 17C | slavonian oak | 99.871 | indigenous oak | 0.129 |
| Kanitz 32H/39A | slavonian oak | 100.000 | indigenous oak | 0.000 |

Table S. 6 Results of the GeneClass analysis based on Nei's standard (Nei 1972) distance, distance of Goldstein et al. (1995) and Bayesian (Baudouin und Lebrun 2001) after exclusion of indigenous haplotypes.

| **Nei's standard distance** | rank 1 | score % | rank 2 | score % | indigenous oak distance | Slavonian oak distance |
|---|---|---|---|---|---|---|
| Tomberg 10B2 | Slavonian oak | 58.817 | indigenous oak | 41.183 | 0.034 | 0.024 |
| Freiherr v. der Leyen 17C | Slavonian oak | 57.241 | indigenous oak | 42.759 | 0.030 | 0.023 |

| **Goldstein** | rank 1 | score % | rank 2 | score % | indigenous oak distance | Slavonian oak distance |
|---|---|---|---|---|---|---|
| Tomberg 10B2 | Slavonian oak | 58.417 | indigenous oak | 41.583 | 0.910 | 0.648 |
| Freiherr v. der Leyen 17C | Slavonian oak | 77.725 | indigenous oak | 22.275 | 1.217 | 0.349 |

| **Bayesian** | rank 1 | score % | rank 2 | score % |
|---|---|---|---|---|
| Tomberg 10B2 | Slavonian oak | 100 | indigenous oak | 0 |
| Freiherr v. der Leyen 17C | Slavonian oak | 100 | indigenous oak | 0 |

Table S. 7 Summary of the genetic parameters and null alleles per loci. agSSRs developed from *Q. rubra*, bEST-SSRs developed for *Q. rubra*, without sign=EST-SSRs developed for *Q. robur*.

| mean across all populations | 2P24[a] | 3A05[a] | 3D15[a] | FIR013 | FIR028 | FIR035 | GOT040 | Pie125 | VIT023 | VIT107 |
|---|---|---|---|---|---|---|---|---|---|---|
| **N** | 18.6 | 18.6 | 18.5 | 18.6 | 18.6 | 18.6 | 18.6 | 18.6 | 18.6 | 18.6 |
| **$N_a$** | 2.541 | 1.946 | 2.838 | 3.892 | 6.703 | 7.432 | 9.378 | 4.865 | 2.486 | 11.351 |
| **$H_o$** | 0.109 | 0.115 | 0.307 | 0.700 | 0.540 | 0.571 | 0.837 | 0.649 | 0.186 | 0.767 |
| **$H_e$** | 0.120 | 0.117 | 0.363 | 0.664 | 0.768 | 0.676 | 0.832 | 0.668 | 0.180 | 0.863 |
| **$F_{ST}$** | 0.038 | 0.029 | 0.040 | 0.033 | 0.056 | 0.051 | 0.036 | 0.062 | 0.025 | 0.048 |
| **Frequency of null alleles** | - | - | 0.446 | - | 0.126 | 0.641 | - | 0.01 | 0.006 | 0.032 |
| **Frequency of null alleles across loci** | | | 0.015 | | | | | | | |

| mean across all populations | FIR104 | Pie102 | Pie267 | Qr0057[b] | Qr0332[b] | FS_C1423[b] | FS_C2361[b] | FS_C2660[b] | FS_C2791[b] | FS_C8183[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| **N** | 18.6 | 18.6 | 18.6 | 18.6 | 18.6 | 18.5 | 18.6 | 18.5 | 18.5 | 18.6 |
| **$N_a$** | 5.703 | 6.243 | 8.784 | 3.892 | 3.649 | 3.514 | 4.432 | 4.865 | 2.216 | 3.054 |
| **$H_o$** | 0.685 | 0.515 | 0.762 | 0.499 | 0.575 | 0.283 | 0.557 | 0.656 | 0.235 | 0.245 |
| **$H_e$** | 0.696 | 0.553 | 0.794 | 0.542 | 0.583 | 0.270 | 0.505 | 0.635 | 0.232 | 0.261 |
| **$F_{ST}$** | 0.039 | 0.036 | 0.036 | 0.041 | 0.034 | 0.033 | 0.044 | 0.048 | 0.048 | 0.037 |
| **Frequency of null alleles** | 0.013 | 0.034 | 0.010 | 0.028 | 0.010 | 0.005 | - | - | - | 0.005 |
| **Frequency of null alleles across loci** | | | 0.029 | | | | | | | 0.007 |

Table S. 8 Bottleneck results based on Wilcoxon's signed-rank test (one-tailed=W_1t). No values are significant at $\alpha$=0.05. N=population size; k=number of alleles; $H_e$=heterozygosity; IAM=infinite alleles model; SSM=stepwise mutation model

| Population | mean_N | mean_k | mean_He | p_W_1t_ IAM | p_W_1t_ SMM | Allele frequency distribution |
|---|---|---|---|---|---|---|
| **Arnsberg 336B** | 40.00 | 4.80 | 0.5099 | 0.4765 | 0.9998 | L-shaped |
| **Studienfond Münster 11C** | 39.60 | 4.55 | 0.5157 | 0.3506 | 0.9914 | L-shaped |
| **Westerholt, Freckenh. 308B/309A** | 40.00 | 5.15 | 0.5129 | 0.6629 | 0.9999 | L-shaped |
| **Graf Merveldt 3A** | 40.00 | 4.15 | 0.4810 | 0.2974 | 0.9953 | L-shaped |
| **Schulze Pellengahr 2A** | 32.00 | 4.65 | 0.4943 | 0.6746 | 0.9988 | L-shaped |
| **Kottenforst 85D** | 38.00 | 4.70 | 0.5318 | 0.1012 | 0.9893 | L-shaped |
| **Kottenforst 85B** | 34.00 | 4.95 | 0.5322 | 0.3542 | 0.9973 | L-shaped |
| **Kottenforst 134A&C** | 40.00 | 5.40 | 0.5599 | 0.2729 | 0.9972 | L-shaped |
| **Kottenforst 40B** | 38.00 | 4.95 | 0.5290 | 0.4347 | 0.9992 | L-shaped |
| **Kanitz 76 A** | 40.00 | 5.10 | 0.5496 | 0.1387 | 0.9819 | L-shaped |
| **Gut Ulenburg 4C** | 39.90 | 5.05 | 0.5475 | 0.3371 | 0.9996 | L-shaped |
| **Rhede 105/4A1/A7** | 40.00 | 5.25 | 0.5105 | 0.8058 | 0.9999 | L-shaped |
| **Kirche Anröchten 32C** | 38.00 | 5.35 | 0.5524 | 0.3506 | 0.9993 | L-shaped |
| **Nagel-Doornick 50B** | 31.10 | 4.80 | 0.5415 | 0.2729 | 0.9867 | L-shaped |
| **Nagel-Doornick 36E** | 31.90 | 5.15 | 0.5461 | 0.4636 | 0.9999 | L-shaped |
| **Schulze-Sutthoff Flur 2/70** | 31.80 | 5.05 | 0.5345 | 0.7625 | 0.9990 | L-shaped |
| **Hamm-Osttünnen 1B1a** | 32.00 | 5.10 | 0.5256 | 0.2839 | 0.9820 | L-shaped |
| **Hamm-Osttünnen 1B1b** | 32.00 | 4.95 | 0.5380 | 0.2608 | 0.9819 | L-shaped |
| **Nagel-Doornick 33C** | 32.00 | 4.75 | 0.5374 | 0.0616 | 0.9669 | L-shaped |
| **Königsforst 127c** | 40.00 | 5.10 | 0.5403 | 0.2979 | 0.9932 | L-shaped |
| **Tomberg 10B2** | 40.00 | 5.20 | 0.5565 | 0.2045 | 0.9867 | L-shaped |
| **Kottenforst 70D** | 39.90 | 4.90 | 0.5286 | 0.2045 | 0.9914 | L-shaped |
| **Königsforst 76B** | 40.00 | 5.40 | 0.5278 | 0.4782 | 0.9953 | L-shaped |
| **Stadt Viersen 36B/38** | 40.00 | 5.10 | 0.5335 | 0.1650 | 0.9800 | L-shaped |
| **Blix Flur 2/155** | 40.00 | 4.75 | 0.5032 | 0.2152 | 0.9852 | L-shaped |
| **Kanitz 77C** | 40.00 | 5.20 | 0.5374 | 0.1387 | 0.9836 | L-shaped |
| **Schulze-Becking 54B1/B2** | 40.00 | 4.95 | 0.5245 | 0.1227 | 0.9709 | L-shaped |
| **Vinkovsi** | 40.00 | 5.10 | 0.5122 | 0.3371 | 0.9999 | L-shaped |
| **Freiherr v. der Leyen 17C** | 39.60 | 5.55 | 0.5587 | 0.7392 | 0.9979 | L-shaped |
| **Plettenberg 106G** | 38.00 | 5.65 | 0.5810 | 0.4492 | 0.9880 | L-shaped |
| **Schulze Pellengahr 4H** | 40.00 | 4.90 | 0.5358 | 0.1012 | 0.9734 | L-shaped |
| **Kanitz 19 A** | 40.00 | 5.25 | 0.5100 | 0.5938 | 0.9996 | L-shaped |
| **Kanitz 32H/39A** | 40.00 | 5.15 | 0.5437 | 0.1387 | 0.8773 | L-shaped |
| **Plettenberg 104 A** | 40.00 | 5.15 | 0.5330 | 0.2608 | 0.9819 | L-shaped |
| **Steprath 3H** | 40.00 | 5.35 | 0.5626 | 0.0909 | 0.9332 | L-shaped |
| **Estermann 116 A1** | 40.00 | 5.05 | 0.5171 | 0.4347 | 0.9904 | L-shaped |
| **Kottenforst 154B** | 8.00 | 3.00 | 0.5143 | 0.2585 | 0.6944 | shifted mode |

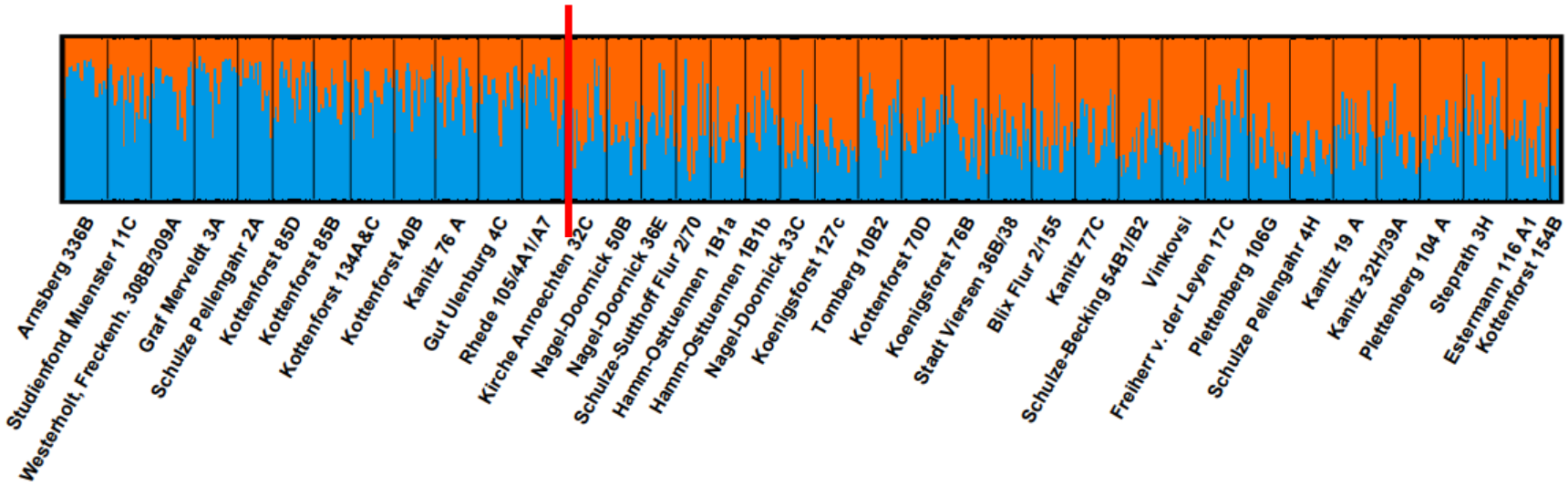


**Figure S. 8** STRUCTURE analysis with admixed model without LOCPRIOR for K=2. Orange=Slavonian oak, blue=common oak. The red line divides the indigenous oaks (left) from the Slavonian oaks (right).

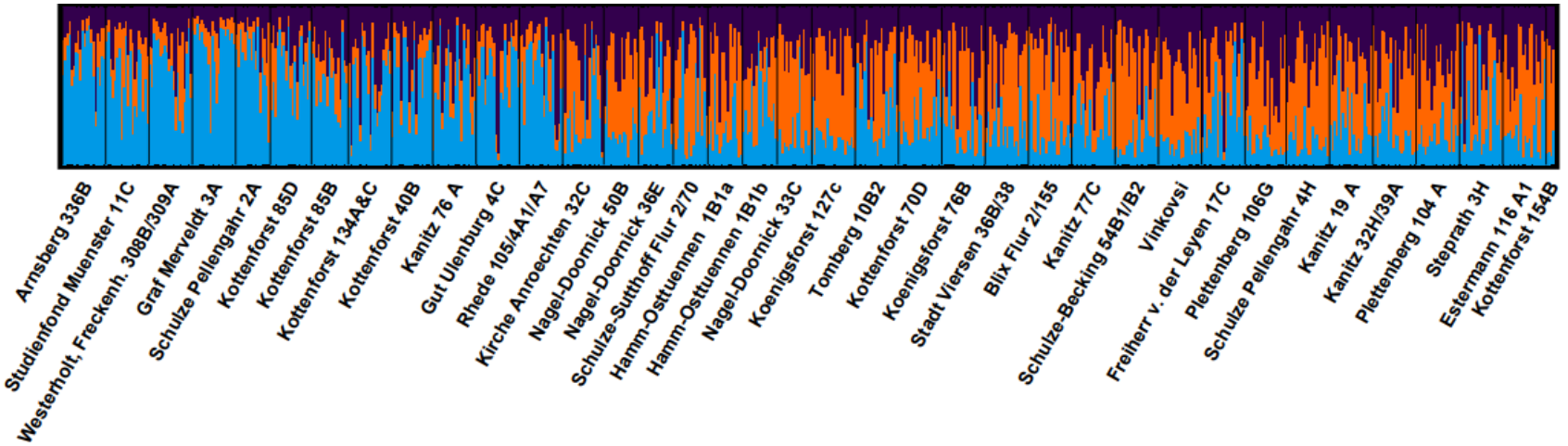


**Figure S. 9** STRUCTURE analysis with admixed model without LOCPRIOR for K=3.

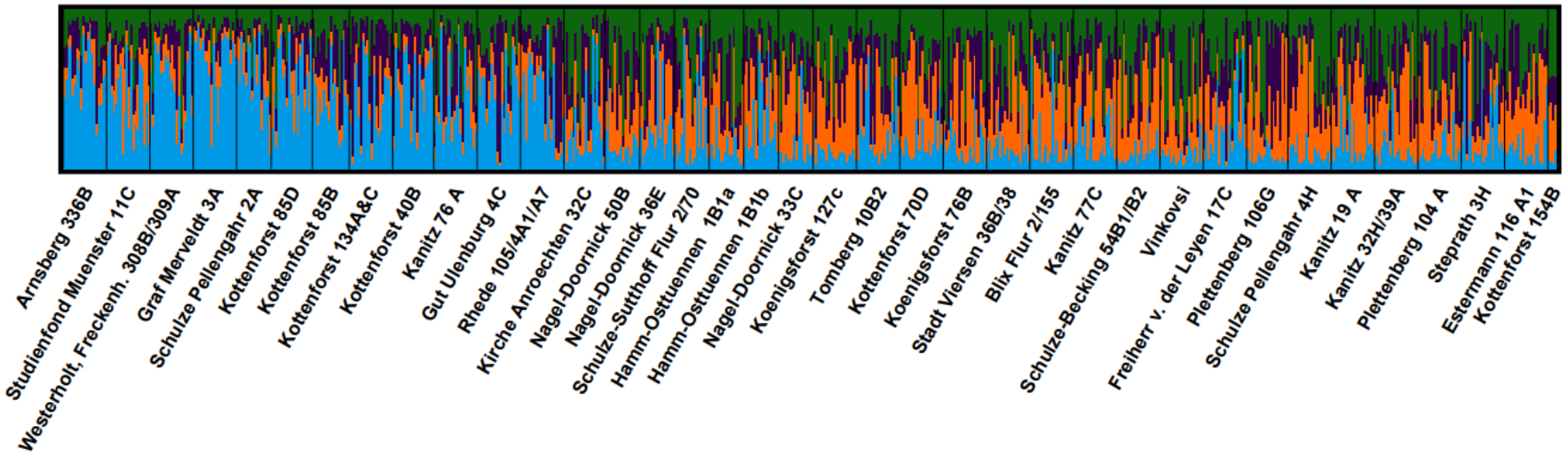


Figure S. 10 STRUCTURE analysis with admixed model without LOCPRIOR for K=4.

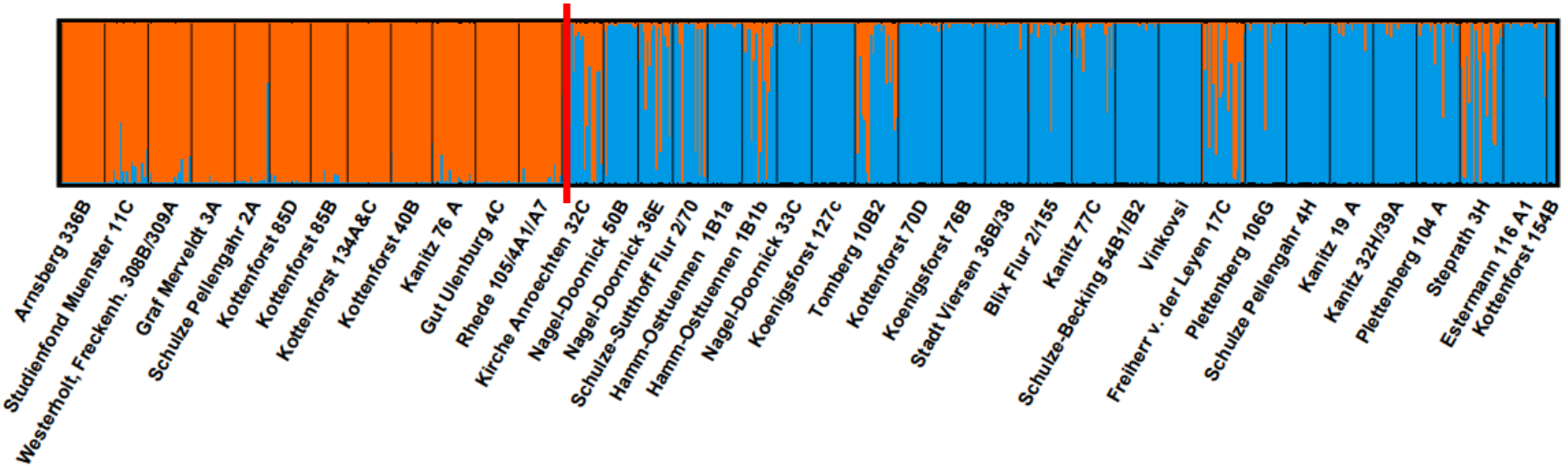


Figure S. 11 STRUCTURE analysis with “no admixture” model and LOCPRIOR for K=2. Blue=Slavonian oak, Orange=common oak. The red line divides the indigenous oaks (left) from the Slavonian oaks (right).

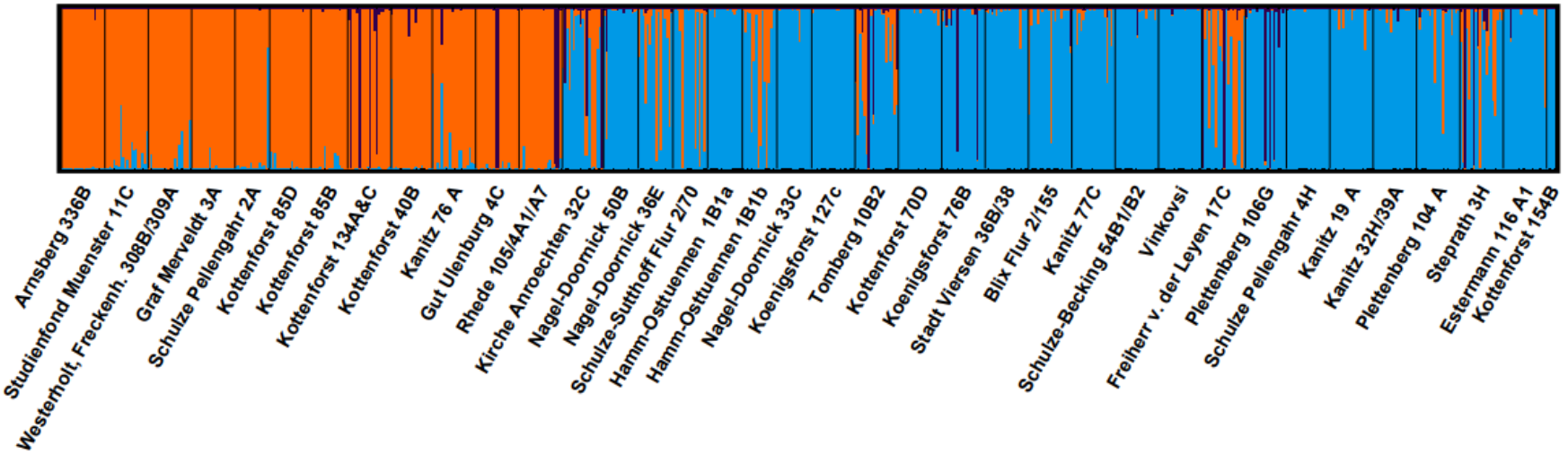


**Figure S. 12** STRUCTURE analysis with "no admixture" model and LOCPRIOR for K=3.

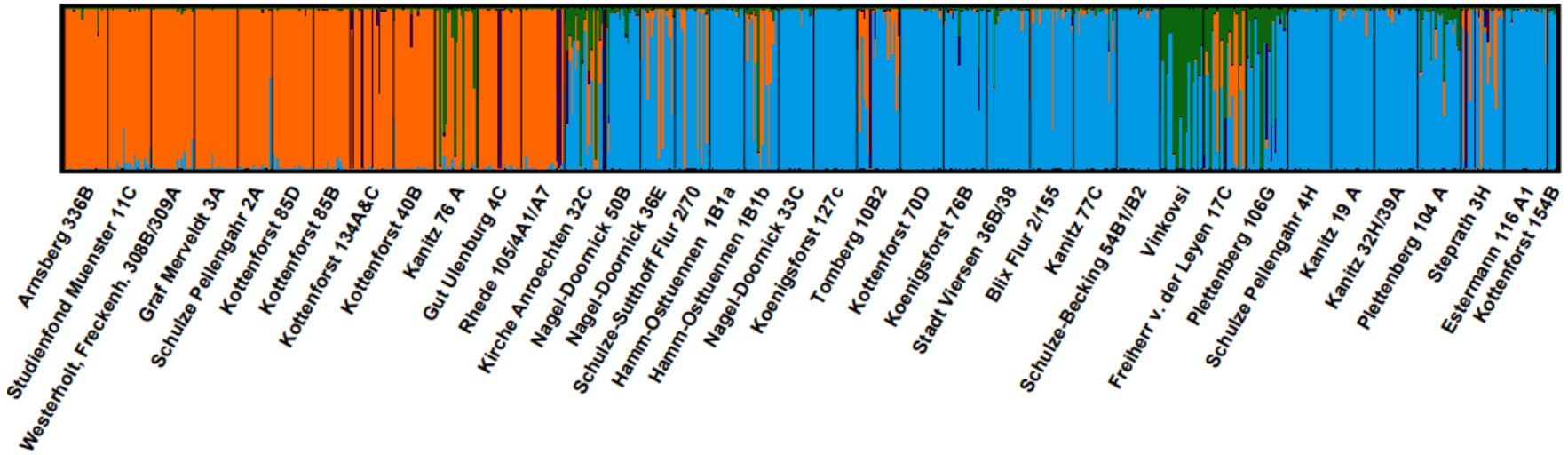


**Figure S. 13** STRUCTURE analysis with "no admixture" and LOCPRIOR model for K=4.

Kapitel 4

# Genetic variability of indigenous (*Quercus robur* L.) and late flushing oak (*Quercus robur* L. subsp. *slavonica* (Gáyer) Mátyás) in adult stands compared with their natural regeneration

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# Genetic variability of indigenous (*Quercus robur* L.) and late flushing oak (*Quercus robur* L. subsp. *slavonica* (Gáyer) Mátyás) in adult stands compared with their natural regeneration

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**Author contributions** KB wrote the main manuscript text and prepared all figures and tables. OG revised it critically for important intellectual content. All authors reviewed the manuscript.

## Abstract

Slavonian oak (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) is currently gaining interest in forestry due to forest restructuring in Germany caused by climate change. Slavonian oaks originating from Croatia have been introduced into Germany mainly in the Münsterland region of North Rhine-Westphalia since the second half of the nineteenth century. They are characterised by their late bud burst, long clear bole, stem straightness and faster height and diameter growth compared to indigenous oaks in Germany. In this study, the genetic differentiation of adult trees and their respective progeny of two Slavonian and two indigenous stands in Hamm-Westtünnen, was evaluated. Genetic diversity and structure were estimated using 23 nuclear simple sequence repeat (SSRs) and 5 maternally inherited chloroplast microsatellite markers (cpSSRs). The mean expected heterozygosity of 0.545 and allelic richness of 6.23 indicate high genetic diversity in the studied populations. The group of progenies ($A_R$=8.40, $H_o$=0.524, $H_e$=0.559, $F_{IS}$=0.064) shows similar levels of genetic variation as the adult stands ($A_R$=8.37, $H_o$=0.513, $H_e$=0.554, $F_{IS}$=0.075). The genetic differentiation between adult stands and progeny was low ($F_{ST}$=0.013). Genetic assignment of individuals using STRUCTURE revealed that the studied populations were divided into two clusters. There was no evidence of extensive hybridization or gene flow between Slavonian and native populations, possibly due to the different timing of bud burst of the two taxa.


**Key words**: nSSR markers, gene flow, Slavonian oak, *Quercus robur* L. subsp. *slavonica*, genetic differentiation, chloroplast microsatellites

## 4.1 Introduction

In addition to forest fragmentation and urbanization, more frequent extreme weather events and the associated spread of pests and diseases, as well as the maladaptation of tree populations that have been translocated to new sites, threaten existing forests (Ivetić et al. 2016). Due to global warming and associated temperature extremes, forest trees will have to adapt to the major climate changes in the future. Thus, translocated populations also offer the possibility to study adaptation processes and to evaluate their suitability in the face of climate change. The adaptability of a plant is determined by its genetic material and by environmental factors at its growth site (Finkeldey and Hattemer 2010). A prerequisite for both this adaptation and for maintaining adaptive capacity for future generations is sufficient genetic variation in populations (Vornam et al. 2004, Gailing et al. 2008). The conservation of genetic variation plays an important role in forestry in the context of restructuring towards more climate-stable forests in order to sustain vital and productive stands. Therefore, the response of forest trees to climate change is determined by the interplay of genetic variation, adaptation, gene flow, and interspecific hybridization (Kremer 2010).

White oaks (section *Quercus*, family Fagaceae) are dominant and diverse forest tree species widely distributed across the Northern Hemisphere (Nixon 1993, Aldrich and Cavender-Bares 2011, Leroy et al. 2020). With regard to climate change, it is assumed that oaks will play an increasingly important role in forests, as they have a high adaptation potential as well as drought tolerance and could thus respond positively to a deficit in the climatic water balance. Due to their major ecological as well as economic significance, and their propensity for hybridization and gene flow (e.g. Burger 1975, Rushton 1993, Lepais et al. 2009, Kremer and Hipp 2020) oaks have been intensively studied, particularly with regard to their genetic diversity and especially their geographic structure (Dumolin et al. 1995, Curtu et al. 2007, Aldrich and Cavender-Bares 2011).

Pedunculate oak (*Quercus robur* L.) is one of the dominant oak species in Germany as well as most of Europe (Barreneche et al. 1998, Reif et al. 2016). Within the species *Quercus robur*, numerous taxonomic subspecies or ecological varieties have evolved over time in geographic sub-areas throughout its range, such as *Quercus robur* subsp. *slavonica* (Gáyer) Mátyás with distribution in Slavonia (eastern region of Croatia) (Burger and Gailing 2021). Slavonian oak is in demand as imported timber throughout Europe, mainly because of its very good qualities (Rieger 2018), and is currently gaining interest for forestry due to forest restructuring in Germany caused by climate change (Burger and Gailing 2021). Especially from a yield point of view, it has a great number of remarkable characteristics like high growth performance, straightness, a long clear bole as well as fine branches (Wachter 2001, Gailing et al. 2003). Slavonian oaks have been introduced into the western part of Germany, especially in the region around Münster, in the second half of the nineteenth century with

the beginning of extensive seed trade through steam engines (Wachter 2001, Gailing et al. 2007a). Accordingly, the Slavonian oak stands are first generation stands in Germany.

Over the last two decades, the development and accessibility of several molecular markers enabled precise characterization of forest genetic resources (different allele frequencies) as well as the study of hybridization and gene flow among oak species (Crăciunesc et al. 2013, Lind and Gailing 2013). For instance, nuclear microsatellites (nSSRs) can be used to study genetic differentiation and structures as well as gene flow between related species (e.g., Lexer et al. 2005, Lepais et al. 2009, Lind and Gailing 2013, Pérez-Pedraza et al. 2021), and chloroplast DNA markers (cpSSRs) for studying postglacial recolonization patterns (e.g., Petit et al. 2002b) in order to describe past episodes of introgression.

Here, we employed molecular markers (nSSRs and cpSSRs) to examine the genetic variation in adult stands and their natural regeneration and the presence of gene flow between populations of *Quercus robur* subsp. *robur* and *Quercus robur* subsp. *slavonic*a. The present paper will address the following questions: (i) Is there any evidence of gene flow between the two closely related oak taxa, based on genetic assignment analysis comparing adult and offspring generations? (ii) Is the haplotype composition of the natural regeneration in accordance with the one of the adult stands? (iii) Does the amount of genetic variation vary among varieties and their natural regeneration?

## 4.2 Materials and Methods

### 4.2.1 Plant material and DNA isolation

Plant material (leaves) of in total 270 trees from four neighboring stands (SLAV_161 A2, SLAV_161 A1, IND_160 B, IND_159 B) in Hüls (Hamm-Westtünnen, NRW) owned by Freiherr v. Boeselager was collected by shooting branch tips in early July 2019 (see Fig. 1, Table 1). Samples for SLAV_161 A2, SLAV_161 A1 and IND_160 B were randomly distributed across the plots, while for IND_159 B only the northern part of the plots were sampled (Figure 1). Due to topographical and local conditions, as well as a very heterogeneous inventory picture, the northern part of 159 B was regarded as an independent stand from the rest of the entire stand. The stands are between 125 and 133 years old (Table 1). In addition, leaves of 369 seedlings from the natural regeneration were sampled in all stands at 7-12 sample points evenly distributed throughout the sampled part of each stand. Gailing et al. (2003) characterized the four stands based on 10 individuals at chloroplast-DNA-markers and classified them at the tree level into early, intermediate, and late flushing according to Wachter (2001) (Table 1). The stands IND_159 B and IND_160 B showed an early bud burst and also the indigenous haplotype 1 (only one tree in IND_159 B showed haplotype 2), while the trees in the stand SLAV_161 A1 consistently sprouted later (Gailing et al. 2003). Stand SLAV_161 A1 was characterized by the Slavonian haplotype 2 (8 individuals) and haplotype 5 (two individuals, haplotype present in both Germany and Croatia). Stand SLAV_161 A2 showed a heterogeneous picture with early and late sprouting trees (intermediate)

and was characterized by the haplotypes HP2 (4 individuals), HP5 (4 individuals) and HP10 (two individuals) (Table 1). Based on morphological and phenological classification of stands according to Wachter (2001) and genotyping with chloroplast markers by Gailing et al. (2003), two indigenous (IND_159 B, IND_160B) and two Slavonian (SLAV_161 A1, SLAV_161 A2) were identified.

The genomic DNA was extracted from in total 749 freshly collected leaves (approx. 1 $cm^2$ piece of leaf) using the DNeasy 96 Plant Kit from Qiagen (Hilden, Germany). Subsequently, the amount of DNA was checked on 1 % agarose gels.

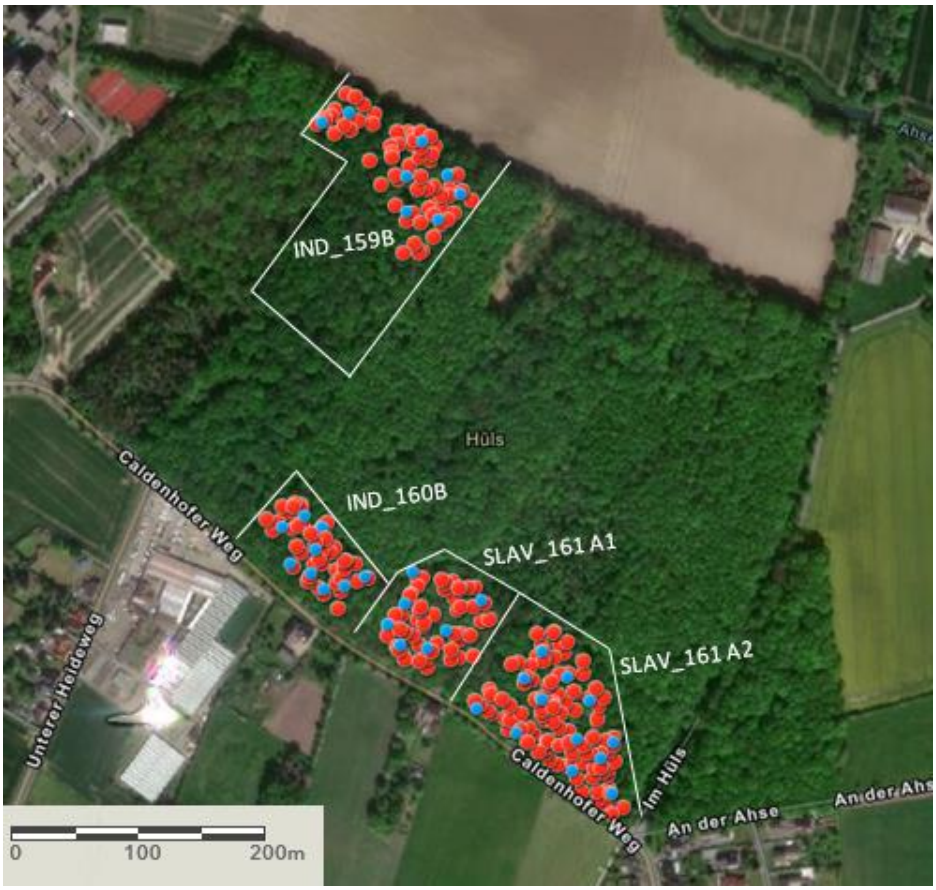


Figure 1 Location of the four stands in Hüls (Hamm-Westtünnen, NRW) with all sampled trees (red dots) and the sampling points of natural regeneration (blue dots). White lines are the borders of all stands. IND=indigenous stand, SLAV=Slavonian stand. Map created in ArcGIS Online (Esri, California, USA).

Table 1 Description of the Slavonian (SLAV) and indigenous (IND) stands in Hüls (Hamm-Westtünnen, NRW).

| Stand | Latitude | Longitude | Area (ha) | Age (years) | Bud burst | References |
|---|---|---|---|---|---|---|
| SLAV_161 A2 | N 51° 39' 39.0" | E 7° 51' 48.9" | 1.43 | 133 | intermediate | Gailing et al. 2003 |
| SLAV_161 A1 | N 51° 39' 43.6" | E 7° 51' 40.0" | 1.21 | 125 | late | Gailing et al. 2003 |
| IND_159 B | N 51° 39' 56.6" | E 7° 51' 39.6" | 3.28 | 125 | early | Gailing et al. 2003 |
| IND_160 B | N 51° 39' 46.3" | E 7° 51' 34.5" | 2.02 | 125 | early | Gailing et al. 2003 |

### 4.2.2 Nuclear microsatellites

For the genotyping with nuclear microsatellites one genomic simple sequence repeat (Aldrich et al. 2002), three genomic simple sequence repeats (gSSRs) (Sullivan et al. 2013) and 19 gene-based expressed sequence tag–simple sequence repeats (EST-SSRs) (Durand et al. 2010, Burger et al. 2018, Müller and Gailing 2018) were used (Table 2).

Thereof, 11 primer pairs were originally developed for *Quercus rubra*, of which 7 were derived from a *Quercus rubra* EST-library (https://www.hardwoodgenomics.org/Transcriptome-assembly/1963023?tripal_pane=group_description_download) and the other four markers were from Aldrich et al. (2002) and Sullivan et al. (2013). The other 12 markers were originally developed for *Quercus robur* in Durand et al. (2010). We chose these 23 markers because of the high transferability of EST-SSRs between *Quercus* species, and the availability of a large number of nSSRs and EST-SSRs developed for *Q. robur*, *Q. petraea*, and related species (Steinkellner et al. 1997, Barreneche et al. 1998, Tanaka et al. 1999, Durand et al. 2010) that have been used in previous studies on indigenous and Slavonian oaks (e.g., Burger et al. 2021). The annotation of the sequences was obtained by searching the individual primer sequences in the respective contigs to identify the complete contig sequences for similarity searches against the UniProt Viridiplantae database (The UniProt Consortium 2017) using BLASTx (Basic Local Alignment Search Tool) (Altschul et al. 1990).

**Table 2** Primer sequences and description of nuclear microsatellites.

| Developed for | Primer name | Repeat motif | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') | Size (bp) | Annotation of sequences |
|---|---|---|---|---|---|---|
| *Quercus rubra*[a] | quru_GA-OC11 | $(GA)_{15}$ | ATACCCAGCTCCCATGACCA | TTTGATTGATATAATTGATCGCT | 195–229 | – |
| *Quercus rubra*[b] | 2P24 | $(CA)_{14}$ | GCAAGAGATCACACACAAACTAGC | CTTTGGGTTCACCAAACAGC | 131–162 | – |
| *Quercus rubra*[b] | 3A05 | $(CA)_{12}(CT)_2$ | AACGTGACCTCTCTCACAGC | AGTGCTGGAGTGCTCATGG | 139–162 | – |
| *Quercus rubra*[b] | 3D15 | $(CA)_{15}$ | GGTGGTGGCAGATACACTGG | GACTCAGACAACCAACTTCAGG | 210–217 | – |
| *Quercus robur* | FIR013 | $(CAG)_5$ | CGGGGAGGTTGATGAGTATT | AACACTGTCACCCCCATAGC | 129–150 | Constans 1 |
| *Quercus robur* | FIR028 | $(TC)_8$ | GGAAGAGTGTTCGGAAAGCA | CCAGCTCCTCCACAATAGCA | 199–215 | tropinone reductase homolog at1g07440 |
| *Quercus robur* | FIR035 | $(AT)_6$ | GCTAAGGTTCCGTGTTCCAA | GGCCAGCAACTAAACCAAGA | 146–177 | chaperone protein dnaj |
| *Quercus robur* | FIR043 | $(TC)_9$ | TTCTCCATTTCACACGCTTC | ACGACATCGTTTTGGAGCTT | 108–133 | r2r3myb transcription factor |
| *Quercus robur* | PIE040 | $(TTC)_8$ | GTGAGAGAGAGAGAGACAAAGAAGAAAAA | AAATTCTCCGCCACATTGAG | 165–188 | basic leucine zipper transcription factor-like protein |
| *Quercus robur* | PIE125 | $(GGAAGC)_3$ | AATACAAATCGCAGGAGGTG | CTAACCCATCGTTCATGGAG | 139–180 | dnaj-like protein |
| *Quercus robur* | GOT040 | $(GA)_{11}$ | AAGGCACTCGTCGCTTTCTA | ACCGATTTGAAGCTCGAGAA | 210–249 | 40s ribosomal protein s16 |
| *Quercus robur* | VIT107 | $(TA)_{13}$ | TGATCACAGATTGGAGCTTAACA | CCCCCACTTAGGAAAGAAGC | 121–161 | light-harvesting complex i protein lhca2 |
| *Quercus robur* | VIT023 | $(ATA)_6$ | AATGCGAACGACATGAACAA | CTCTCGTCGGAGACTCAACC | 117–133 | ap2 erf domain-containing transcription factor |
| *Quercus robur* | FIR104 | $(GGT)_7$ | TTAACTCGGTTTGCGACTCA | AGCACGTGACTCGACCTGTA | 198–221 | r2r3-myb transcription factor |
| *Quercus robur* | PIE102 | $(AG)_{12}$ | ACCTTCCATGCTCAAAGATG | GCTGGTGATACAAGTGTTTGG | 138–167 | TC58546 similar to UP\|Q8NQE3_CORGL Predicted transcriptional regulator, partial |
| *Quercus robur* | PIE267 | $(AG)_{11}$ | CCAACCATCAAGGCCATTAC | GTGCGAACAGATCCCTTGTC | 74–114 | – |
| *Quercus rubra*[c] | Qr0057 | $(AAG)_7$ | CCGACCTTGTTGATTGTTCC | TATTGATCCTATCGGAGGCG | 125–153 | Glycoprotein gp2 |
| *Quercus rubra*[c] | Qr0332 | $(CCT)_5$ | AATATCAAATCGGCCAGCAG | GTGGTGGACCTGTGCCATAC | 150–175 | Keratin, ultra-high-sulfur matrix protein |
| *Quercus rubra*[c] | Qr1423 | $(CAC)_6$ | TCCCTTCTCGTTTCACCATC | TGCACCATACGGATTGAAAG | 261–303 | Epstein-Barr nuclear antigen 1 |
| *Quercus rubra*[d] | FS_C2361 | $(GAA)_8$ | AGGTCCTTCAGTTTGGGAGC | ATTCCCATGCATCAAAATCC | 185–240 | Light-harvesting complex-like protein OH2 |
| *Quercus rubra*[d] | FS_C2660 | $(GAG)_8$ | AGCAGAATTCGCCAAGTGAT | TGCCTTTGCATTCTCCTCTT | 218–240 | Eukaryotic translation initiation factor 5B-like |
| *Quercus rubra*[d] | FS_C2791 | $(GA)_5$ | CGAAACAGAGAGAACCCAAGA | CTTCAAACATCCAGCGTTGA | 287–298 | 50S ribosomal protein L13, chloroplastic |
| *Quercus rubra*[d] | FS_C8183 | $(AGC)_6$ | TATTCAACCACAGCTGCCTG | ACAGCTGCCTCTGTGGATCT | 200–213 | Auxin response factor 19-like |

[a] SSRs developed in Aldrich et al. (2002); [b] gSSR developed in Sullivan et al. (2013); [c] Müller and Gailing (2018) and [d] Burger et al. (2018), derived from a *Q. rubra* EST-library (https://www.hardwoodgenomics.org/Transcriptome-assembly/1963023?tripal_pane=group_description_download). All other markers are EST-SSRs, which were developed for *Q. petraea* and *Q. robur* by Durand et al. (2010).

We used the same multiplex reactions for PCR as described in Burger et al. (2021) with the exception that 3 additional primers (one in multiplex 2 and two in multiplex 3) were used.

Microsatellite loci from each of the 639 individual tree samples were amplified with six different multiplexes in a 13 µl PCR mix. The PCR mix of multiplex 1 (2P24, 3A05, 3D15) and multiplex 2 (FIR013, FIR028, FIR035, FIR043) consisted of 1.5 µl reaction buffer (containing 0.8 M Tris-HCl and 0.2 M $(NH_4)_2SO_4$), 1.5 µl $MgCl_2$ (25 mM), 1 µl dNTPs (2.5 mM of each dNTP), 0.2 µl HOTFIREPol *Taq* polymerase (Solis BioDyne, Estonia) (5 units·µl$^{-1}$), 5.8 µl $H_2O$, 0.5 µl of each forward primer (5 pmol·µl$^{-1}$), 0.5 µl of each reverse primer (5 pmol·µl$^{-1}$) and 1 µl DNA (ca. 0.6 ng·µl$^{-1}$). The PCR mix of multiplex 3 (PIE040, PIE125, GOT040, OC11, VIT023, VIT107) consisted of 6.5 µl Multiplex *Taq* PCR Master Mix Kit (QIAGEN, Germantown, Maryland, USA, providing a final concentration of 3 mM $MgCl_2$), 2.8 µl $H_2O$, 0.4 µl of each forward and reverse primer PIE125 (5 pmol·µl$^{-1}$), 0.7 µl of each forward and reverse primer VIT107 (5 pmol·µl$^{-1}$), 0.25 µl of each forward and reverse primer VIT023 (5 pmol·µl$^{-1}$) 0.5 µl of each forward and reverse primer GOT040, PIE040, OC11 (5 pmol·µl$^{-1}$) and 1 µl DNA (ca. 0.6 units·µl$^{-1}$). For multiplex 4 (PIE102, FIR104, PIE267), the PCR mix consisted of 1.5 µl reaction buffer (containing 0.8 M Tris-HCl and 0.2 M $(NH_4)_2SO_4$), 1.5 µl $MgCl_2$ (25 mM), 1 µl dNTPs (2.5 mM of each dNTP), 0.2 µl HOTFIREPol *Taq* polymerase (Solis BioDyne, Estonia) (5 units·µl$^{-1}$), 6.2 µl $H_2O$, each 0.5 µl forward and reverse primer PIE102 (5 pmol·µl$^{-1}$) and FIR104 (5 pmol·µl$^{-1}$), 0.3 µl of each forward and reverse primer PIE267 (5 pmol·µl$^{-1}$) and 1 µl DNA (ca. 0.6 ng·µl$^{-1}$). For PCR amplifications of multiplex 5 (FS_C032, FS_C2660, FS_C2791, FS_C8183) and 6 (Qr0057, Qr1423, FS_C2361) a cost-effective tailed-primer approach was used (Schuelke 2000, Kubisiak et al. 2009) consisting of 1.5 µl reaction buffer (containing 0.8 M Tris-HCl and 0.2 M $(NH_4)_2SO_4$), 1.5 µl $MgCl_2$ (25 mM), 1 µl dNTPs (2.5 mM of each dNTP), 0.2 µl HOTFIREPol Taq polymerase (Solis BioDyne, Estonia) (5 units·µl$^{-1}$), 5.5 µl $H_2O$, 0.2 µl M13 (5'-CACGACGTTGTAAAACGAC-3´) (Kubisiak et al. 2009) tailed forward primer (5 pmol·µl$^{-1}$), 0.5 µl PIG-tailed reverse primer (5'-GTTTCTT-3') (5 pmol·µl$^{-1}$) (Brownstein et al. 1996, Schuelke 2000, Kubisiak et al. 2009), 1 µl M13 (6-FAM/HEX) primer (5 pmol·µl$^{-1}$), 5 µl $H_2O$ (5.7 µl $H_2O$ for multiplex 6) and 1 µl DNA (ca. 0.6 ng·µl$^{-1}$).

Each PCR reaction was performed in a Biometra Thermal Cycler (MJ Research PTC 200, Analytik Jena, Germany) using the following touchdown program:

An initial denaturation at 95 °C for 15 min, followed by 10 touchdown cycles at 94 °C for 1 min, 1 min at 60 °C (decreasing 1 °C each cycle) and 1 min at 72 °C, followed by 25 cycles at 94 °C for 1 min, annealing at 50 °C for 1 min and elongation at 72 °C for 1 min, and a final extension step at 72 °C for 20 min. Amplification products were separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, USA) using the GeneScan™ Rox-500 and Liz-500 (only for multiplex 3) size

markers. For the fragment length analysis, multiplexes 5 and 6 were run together. Scoring of alleles was conducted using GeneMapper® version 4.1 (Applied Biosystems, Foster City, USA).

### 4.2.3 Chloroplast microsatellites (cpSSRs)

The chloroplast microsatellites *udt1, ucd4* and *udt4* specifically developed for oaks (Deguilloux et al. 2003) (Table S. 1), were analyzed and amplified in all samples. *Ucd4* and *udt4* were used to differentiate between the main haplotypes (Table S. 2). In addition, *udt1* was used to distinguish the southwestern European chloroplast haplotypes HP10-11 and HP12 from each other. Finally, the universal chloroplast markers *ccmp2* and *ccmp10* (Weising and Gardner 1999) were used for additional validation of 28 samples with haplotype 17 (Table S. 2).

The haplotypes HP5 and HP7-26 showed the same length at all used cpSSRs and could therefore not be distinguished at cpSSRs (Gailing et al. 2007a, 2007b) (Table S. 2). Consequently, we consider them as one haplotype HP5/7-26.

Each PCR reaction of the *udt* and *ucd* cpSSRs was also performed in a Biometra Thermal Cycler (MJ Research PTC 200, Analytik Jena, Germany) using the following touchdown program:

An initial denaturation at 95 °C for 15 min, followed by 8 touchdown cycles at 94 °C for 1 min, 1 min at 53 °C (decreasing 1 °C each cycle) and 1 min at 72 °C, followed by 33 cycles at 94 °C for 1 min, annealing at 45 °C for 1 min and elongation at 72 °C for 1 min, and a final extension step at 72 °C for 20 min. The PCR profile for the universal cpSSRs (*ccmps*) consisted of an initial denaturation at 95 °C for 15 min, followed by 35 cycles at 94 °C for 1 min, a 1 min annealing step at 53 °C, an elongation step at 72 °C for 1 min and a final extension step at 72 °C for 10 min.

The forward primers used for PCR reactions are labelled with the fluorescence dyes 6-FAM or HEX (Table 4). The amplification products were diluted (1:200) and separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, USA) in a multiplex analysis. Fragment sizes were determined with the GeneScan™ Rox-500 size marker using the software GeneMapper® version 4.1 (Applied Biosystems, Foster City, USA).

### 4.2.4 Statistical analysis of genetic diversity and differentiation based on nSSRs

The genetic variation indices observed heterozygosity ($H_o$) and expected heterozygosity ($H_e$), as well as the fixation index $F_{ST}$ and Hedrick's standardized $G_{ST}$ ($G'_{ST}$(Hed)) for individual markers and across all markers were calculated in GenAlEx version 6.51b2 (Peakall and Smouse 2006, Smouse et al. 2017). With the software Fstat version 2.9.4 (Goudet 2003) allelic richness ($A_r$) (here: based on a minimum sample size of 48 individuals) as well as the inbreeding coefficients ($F_{IS}$) (Weir and Cockerham

1984) were determined. Differences in levels of $A_R$, $H_o$, $H_e$ and $F_{IS}$ between adults and progeny were analyzed using the 'comparison among groups of samples test' in Fstat computing 1000 permutations. In addition, significant deviations from zero of $F_{IS}$ were determined after standard Bonferroni correction ($\alpha$=0.05, p< 0.00022) implemented in the software Fstat (Goudet 2003). Linkage disequilibrium (LD) was calculated for each pair of loci in the 8 populations using Genepop version 4.7.2 (Rousset 2008) based on the following settings for the Markov chain: dememorization 10000, batches 100 and iterations per batch 5000.

A dendrogram with UPGMA based on Nei's standard genetic distance $D_S$ (Nei 1972) was calculated using the software program Populations version 1.2.32 (Langella 1999), with bootstrap values based on 1000 permutations across loci. Applying the software TreeView version 1.6.6 (Page 1996), the dendrogram was visualized.

To detect signatures of recent genetic bottlenecks and to investigate whether the introduced seeds established from seeds representing a limited number of seed parents, the software BOTTLENECK version 1.2.02 (Piry et al. 1999) was used. Therefore, we performed a Wilcoxon signed-rank test (one-tailed) for heterozygosity excess for 'the two phase-mutation model' (TPM) and 'the stepwise mutation model' (SMM) and a mode-shift analysis to test for a distortion in the allele frequency distribution.

Genotyping errors due to non-amplified alleles (null alleles) which can lead to overestimates of the inbreeding coefficient ($F_{IS}$) were identified using the Windows®-based software MicroChecker version 2.2.3 (Van Oosterhout et al. 2004). Furthermore, Arlequin version 3.5.2.2 (Excoffier and Lischer 2010) was run with 50,000 simulations of 100 demes per group with the infinite island model based on $F_{ST}$ in order to detect outliers which deviate significantly from the variation and differentiation expected under neutrality among all populations and between the groups of indigenous and Slavonian oaks.

An analysis of molecular variance (AMOVA) was used to analyze population differentiation and performed with GenAlEx using 9999 permutations.

### 4.2.5 Analysis of population structure

Population structure was inferred using model-based cluster analysis implemented in the software STRUCTURE version 2.3.4 (Pritchard et al. 2000) to identify possible subpopulations based on the microsatellite dataset. For this purpose, the admixture model was chosen because of its consideration of the ancestry of admixed individuals, and correlated allele frequencies were selected because of their potential to improve clustering in closely related populations, while using a burn-in period of 50,000, Markov chain Monte Carlo (MCMC) replicates of 100,000, and the LOCPRIOR model. However, we used

additionally the default setting in STRUCTURE 'admixed model without LOCPRIOR' to identify population structure solely based on genetic information. In addition, to determine the "best K" from the logarithmic results and the ΔK method (Evanno et al. 2005), the online software STRUCTURE HARVESTER v. 0.6.94 (Earl and vonHoldt 2012) was used. The CLUMPAK software (Cluster Markov Packager Across K) was applied for post-processing and graphical representation of the results of the model-based population STRUCTURE analysis (Kopelman et al. 2015).

Besides, clustering of populations was analyzed using a principal coordinate analysis (PCoA) with the pairwise, individual-by-individual (*N x N*) genetic distance implemented in GeneAlEx in order to find and plot the major patterns within this dataset (Peakall and Smouse 2006, 2012).

## 4.3 Results

### 4.3.1 Chloroplast microsatellites (cpSSRs) and the frequency of cpDNA haplotypes

A total of 5 haplotypes could be distinguished in the studied populations corresponding to the haplotypes HP1, HP10-11, HP2, HP5/7-26, and HP17 (Petit et al. 2002b). Haplotype 1 is the most frequent haplotype in western Germany and dominant in Central Europe, but completely absent in the Balkan region (Bordács et al. 2002, König et al. 2002, Petit et al. 2002a, b). Its glacial refugium was most likely located in southern Italy (Petit et al. 2002a). The haplotypes HP10-11 show a center of distribution in southwestern and western Europe and had their glacial refugia on the Iberian Peninsula (Petit et al. 2002b). Moreover, these haplotype (HP10-11) are also apparently missing in the Balkan region (Petit et al. 2002a). In addition to these presumably indigenous haplotypes, HP2, HP5/7-26 and HP17 have a center of distribution in the Balkan region with presumed southern Italian origin (Bordács et al. 2002, Petit et al. 2002a, b). Although HP2, HP5/7-26, and HP17 are very frequent in the Balkan region (Petit et al. 2002a), none of them are restricted to this region, but only haplotype 5/7-26 occurs naturally in oak populations in both Germany and the Balkan region.

The haplotype composition of each of the four stands (two Slavonian and two indigenous) and their natural regeneration is summarized in Table 3 and visualized in Figure 2.

**Table 3** Description of frequencies of chloroplast (cp) haplotypes for each population. SLAV=Slavonian population, IND=indigenous population, NR=natural regeneration. The most frequent haplotype in each population is printed in bold.

| | Indigenous haplotype | | Slavonian haplotype | | | |
|---|---|---|---|---|---|---|
| **Population** | **HP1** | **HP10-11** | **HP2** | **HP5/7-26** | **HP17** | **Most frequent** |
| SLAV_161 A2 | 5 | 14 | 28 | **50** | 11 | **HP5/7-26** |
| SLAV_NR_161 A2 | 3 | 8 | 33 | **73** | 3 | **HP5/7-26** |
| SLAV_161 A1 | 0 | 0 | **50** | 1 | 0 | **HP2** |
| SLAV_NR_161 A1 | 0 | 0 | **69** | 1 | 0 | **HP2** |
| IND_159 B | **46** | 0 | 4 | 9 | 2 | **HP1** |
| IND_NR_159 B | **49** | 0 | 10 | 10 | 10 | **HP1** |
| IND_160 B | **49** | 0 | 1 | 0 | 0 | **HP1** |
| IND_NR_160 B | **99** | 0 | 1 | 0 | 0 | **HP1** |

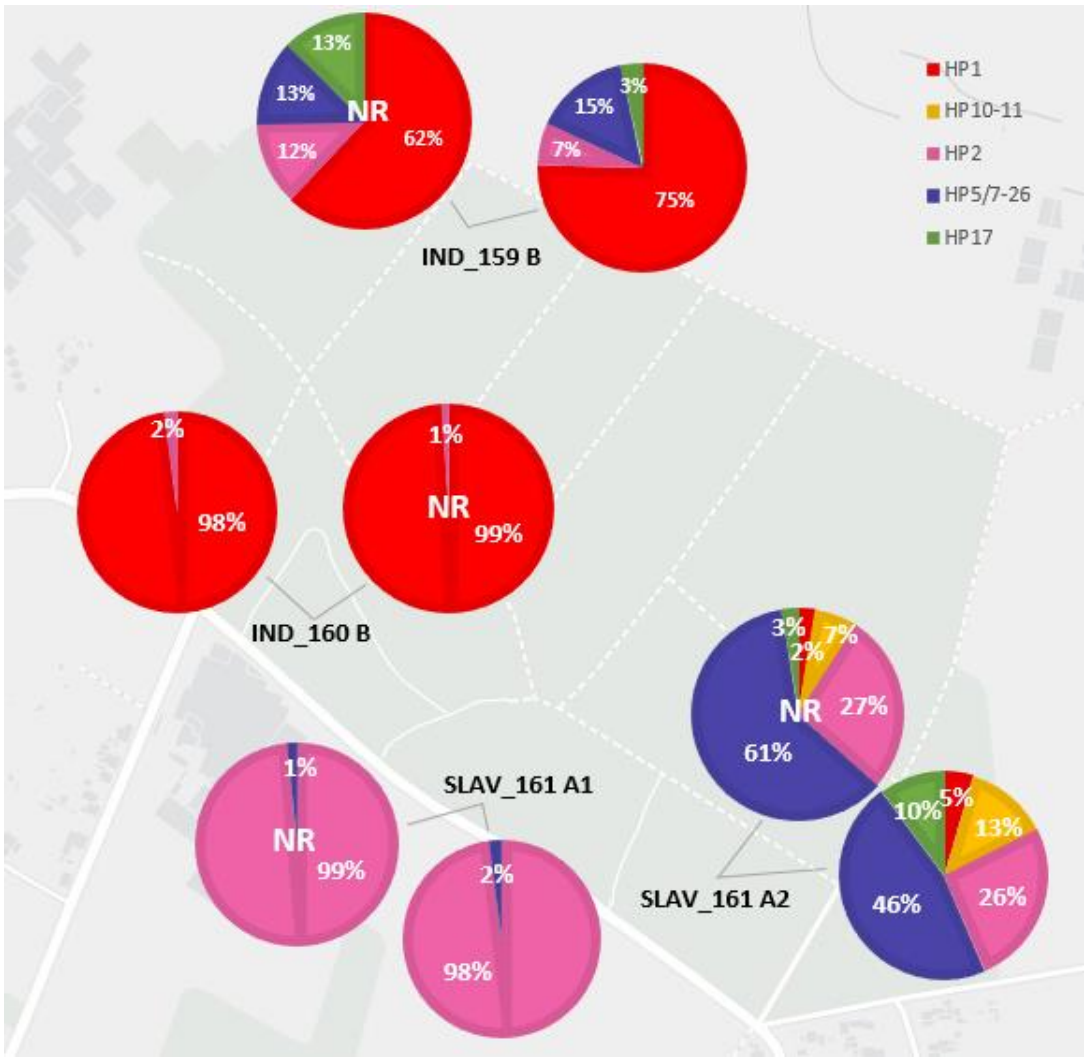


**Figure 2** Haplotype composition of each of the four analyzed stands and their natural regeneration (NR). HP1: Italy-Scandinavia line (lineage C), HP2: Croatia-Sicily line (lineage C), HP5: Italy-Eastern Balkan-Germany line (lineage A), HP7-26: Croatia-Catalonia line (lineage A), HP10: Western Europe-Portugal line (lineage B), HP11: Western Europe line (lineage B), HP17: Italy-Balkan line (lineage E) (as described in Petit et al. (2002b)). SLAV=Slavonian stand, IND=indigenous stand, NR=natural regeneration. Map generated with ArcGIS online.

Stand SLAV_161 A2 consists to 72 % of Slavonian haplotypes with HP5/7-26 as the most frequent one (n=50) followed by HP2 (n=28), HP17 (n=11) and a low proportion of haplotypes HP10-11 (n=14) and HP1 (n=5). All sampled trees of stand SLAV_161 A1 show only Slavonian haplotype 2 except for one tree with HP5/7-26. One of the indigenous stands, IND_160 B, consists to 98 % of HP1 (n=49) and only one individual shows haplotype 2. The other indigenous stand IND_159 B includes only 75 % native oaks with haplotype 1 (n=46) and 25 % with Slavonian haplotype HP2 (n=4), and HP17 (n=2) as well as HP5/7-26 (n=9).

The natural regeneration of SLAV_NR_161 A2, SLAV_NR_161 A1, IND_160 B and IND_159 B shows comparable genetic structures to their adult stands.

### 4.3.2 Variation within and among stands at nuclear microsatellite markers

Inbreeding coefficients across all markers were not significantly different from zero in any population. An overview of the genetic diversity indices (N, $A_R$, $H_o$, $H_e$, $F_{IS}$, $G_{ST}$(Hed), $F_{ST}$) at all 23 markers in the 8 populations can be found in Online-Resource 1. The mean observed heterozygosity ($H_o$) ranged from 0.499 in SLAV_161 A1 to 0.538 in IND_NR_159 B, and the expected heterozygosity ($H_e$) ranged from 0.532 in IND_NR_160 B to 0.556 in IND_159 B and SLAV_NR_161 A2. A summary of the genetic parameters across all loci is given in Table 4.

The mean values for effective number of alleles, allelic richness and inbreeding coefficients (not significant after Bonferroni correction (5 %)) were lower in the natural regeneration ($A_R$: 6.02, $H_e$:0.546, $F_{IS}$: 0.045) compared to adult stands ($A_R$: 6.43, $H_e$:0.545, $F_{IS}$: 0.068). However, the observed and expected heterozygosity was slightly lower in the group of adult stands ($H_o$: 0.513, $H_e$: 0.545) than in the natural regeneration ($H_o$: 0.524, $H_e$: 0.546). The mean observed heterozygosity per locus ranged from 0.092 at locus 2P24 to 0.818 at locus GOT040, and the mean expected heterozygosity per locus ranged from 0.088 at locus 2P24 to 0.879 at locus OC11 (Table S. 3).

The pooled sample of naturally established seedlings across all stands showed no significantly higher (exact test, 1000 permutations, p=0.045) allelic richness ($A_R$) in adult trees (6.43) than in the seedling cohort (6.02; Table 4). Higher levels of $A_R$ in adults compared with seedlings were detected for each population separately. In addition to the mean $A_R$ across populations, observed heterozygosity ($H_o$), expected heterozygosity ($H_e$) and inbreeding coefficient ($F_{IS}$) were also not different between the adult and progeny cohort (exact test, 1000 permutations, $p > 0.05$) (Table 2).

**Table 4** Summary of genetic parameters: N=number of individuals, $A_R$=allelic richness, $H_o$= observed heterozygosity, $H_e$= expected heterozygosity, $F_{IS}$=inbreeding coefficient across all loci. IND=indigenous stand, SLAV=Slavonian stand, NR=natural regeneration.

| Population | N | $A_R$* | $H_o$ | $H_e$ | $F_{IS}$ |
|---|---|---|---|---|---|
| SLAV_161 A2 | 107.00 | 6.72 | 0.509 | 0.548 | 0.076 |
| SLAV_161 A1 | 50.78 | 6.26 | 0.499 | 0.535 | 0.078 |
| IND_160 B | 49.61 | 6.15 | 0.514 | 0.539 | 0.055 |
| IND_159 B | 60.65 | 6.58 | 0.531 | 0.556 | 0.054 |
| **Mean across adult stands** | 67.01 | 6.43[a] | 0.513 | 0.545 | 0.068 |
| **Total variation adult stands** | 306.74 | 8.37 | 0.513 | 0.554 | 0.075 |
| SLAV_NR_161 A2 | 118.87 | 6.51 | 0.524 | 0.556 | 0.062 |
| SLAV_NR_161 A1 | 69.74 | 6.15 | 0.518 | 0.544 | 0.055 |
| IND_NR_160 B | 98.70 | 5.86 | 0.517 | 0.532 | 0.032 |
| IND_NR_159 B | 78.57 | 5.58 | 0.538 | 0.550 | 0.028 |
| **Mean across natural regeneration** | 91.47 | 6.02[a] | 0.524 | 0.546 | 0.045 |
| **Total variation natural regeneration** | 436.17 | 8.40 | 0.524 | 0.559 | 0.064 |
| **Mean across all populations** | 79.24 | 6.23 | 0.519 | 0.545 | 0.059 |
| **Total variation** | 742.91 | 9.08 | 0.520 | 0.560 | 0.072 |

* Allelic richness based on a minimum sample size of 48 individuals; [a] Means across populations are significantly different at the $p < 0.05$ level (1000 permutations, FSTAT).

The genetic differentiation ($F_{ST}$) between the 8 populations (adult and natural regeneration) was relatively low at most loci ranging from 0.009 for FS_C2791 to 0.174 for Qr0332. Overall, the mean differentiation among all populations was 0.022 ($G'_{ST}$(Hed)=0.044) (Online-Resource 1). The mean pairwise $F_{ST}$ between adult stands is 0.010 ($G'_{ST}$(Hed)=0.022), between progeny 0.014 ($G'_{ST}$(Hed)=0.046) and between adult stands and progeny 0.013 ($G'_{ST}$(Hed)=0.038) (Table S. 4). The mean pairwise $F_{ST}$ between indigenous oaks is 0.010 ($G'_{ST}$(Hed)=0.027), between Slavonian oaks 0.009 ($G'_{ST}$(Hed)=0.022) and between indigenous and Slavonian oaks 0.014 ($G'_{ST}$(Hed)=0.042) (Table S. 4). In addition, Table S. 5 shows also the pairwise $F_{ST}$ values between adult and progeny separately for Slavonian and indigenous stands. The low differentiation between adult and offspring generations as well as between indigenous and Slavonian oaks and results of genetic assignment analyses reveal similar genetic structures in both generations and no evidence for extensive gene flow between Slavonian and indigenous stands.

Further, the outlier test with Arlequin showed that there are no markers with signature of selection among populations and between clearly indigenous (IND_160_B) and Slavonian (SLAV_161 A1) stands (Fig. S. 1).

The percentage of all pairs of loci in LD was 0.79 % in the natural regeneration SLAV_NR_161 A2, as well as 0.40 % in the natural regenerations SLAV_NR_161 A1, IND_NR_159 B and IND_NR_160 B (mean over all populations: 0.20 %). All adult populations had no loci in LD.

Null alleles were detected in each of the 10 populations at least at one of the markers OC11, 3D15, FIR028, FIR035, FIR043, GOT040, PIE040 (highest null allele frequencies in IND_NR_159 B: 0.1716, in SLAV_NR_161 A1: 0.1848 and IND_159 B: 0.1716), PIE125, VIT023, VIT107, FS_C2660 and FS_C2791 (highest null allele frequency in SLAV_161 A1: 0.1615) (Table S. 6). However, in all populations null alleles only occurred with a frequency between 0.51 % and 2.51 % over all loci (Table S. 6). Furthermore, null alleles across all populations per locus ranged from 0.006 at locus PIE125 and GOT040 to 0.092 at locus PIE040 (Table S. 3). The comparison of null alleles between marker types showed that two of the four gSSRs (OC11, 3D15) showed null alleles with a low frequency (<0.040), null allele frequencies of EST-SSRs developed for *Q. robur* and *Q. petraea* ranged from 0.006 at locus PIE125 to 0.092 at locus PIE040 and null allele frequencies of EST-SSRs developed for *Q. rubra* ranged from 0.013 at locus Qr0057 and FS_C2660 to 0.016 at locus FS_C2791 (Table S. 3). Across all loci, EST-SSRs developed for *Q. robur* and *Q. petraea* have the highest null allele frequency (0.021), followed by gSSRs (0.012) and EST-SSRs developed for *Q. rubra* (0.006).

The results of the program BOTTLENECK mode-shift analysis showed that the allele frequencies of all populations followed a normal L-shaped distribution as expected for mutation-drift equilibrium (Table S. 7). This may indicate that the introduced seed did not originate from only a limited number of seed parents. The AMOVA overall populations showed that 98 % of the molecular variance was within populations (91 % within and 7 % between Individuals) and only 2 % among populations.

### 4.3.3 Population structure

Using principal coordinate analysis (PCoA), the basic patterns among the populations are presented in Figure 3. Here, the PCoA results do not show a clear differentiation between the indigenous and the Slavonian populations. Even though there is a separation along Axis 1, the natural regeneration of IND_159 B groups with Slavonian stands (Fig. 3, see also Fig. 5). In comparison, PCoA based on chloroplast microsatellite markers shows that each natural regeneration is grouped with their adult stand (Fig. 4).

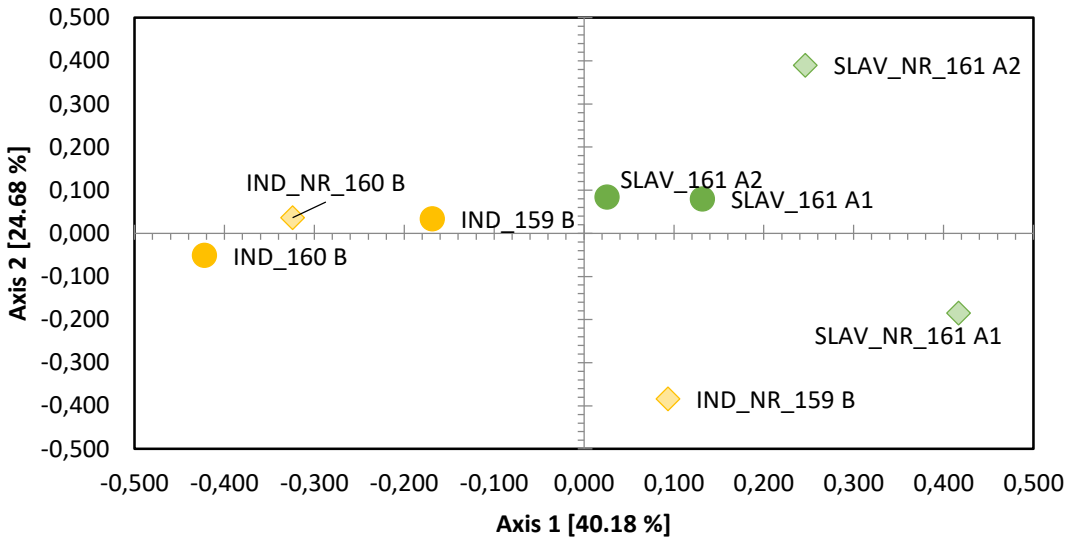


**Figure 3** Principal coordinate analysis (PCoA) based on nuclear microsatellite markers (nSSRs) for all populations. Green and SLAV=Slavonian stand, yellow and IND=indigenous stand, circle=adult stands, square=natural regeneration.

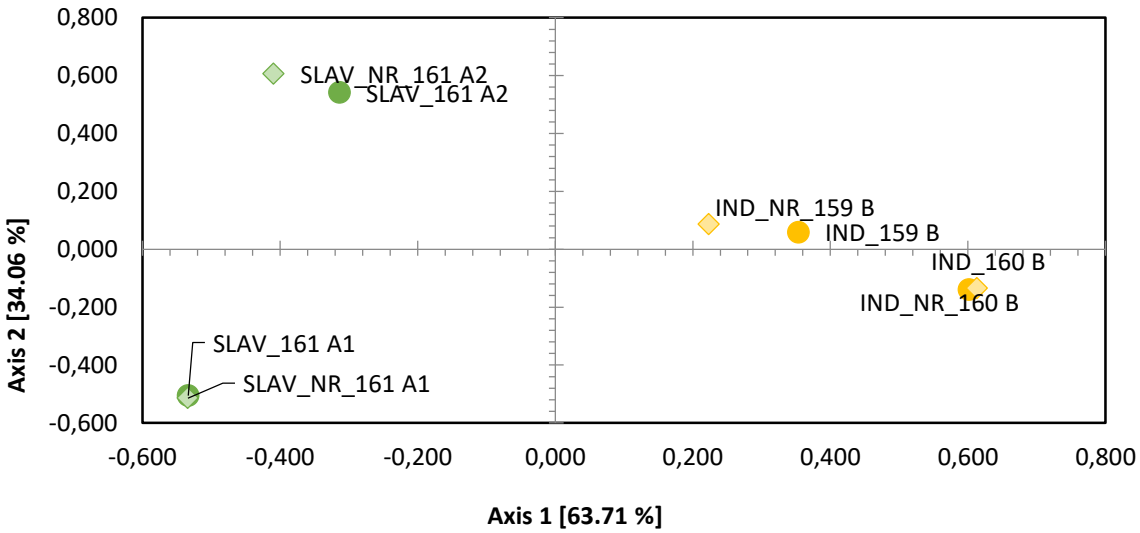


**Figure 4** Principal coordinate analysis (PCoA) based on chloroplast microsatellite markers (cpSSRs) for all populations. Green and SLAV=Slavonian stand, yellow and IND=indigenous stand, circle=adult stands, NR and square=natural regeneration.

Using the Evanno method (Evanno et al. 2005) and STRUCTURE HARVESTER (Earl and vonHoldt 2012) an optimal and significant K=2 was determined (Fig. S. 2, Fig. S. 3 and Table S. 8). The diagram calculated by STRUCTURE using the admixed model and LOCPRIOR with the population assumption of K=2 is presented in Figure 5. In addition, Table 5 shows the proportion of membership of each sampled population in the two clusters, but there is no specific cluster for each origin, since both clusters occur in both origins. A proportion of ancestry > 0.5 or < 0.5 in cluster 1 is characteristic for indigenous and Slavonian stands, respectively. In addition, Figure 6 combines the STRUCTURE results with the haplotypes and shows that the Slavonian haplotypes (HP2, HP5/7-26, HP17) are differentiated from non-Slavonian HP1, HP10-11 at nSSRs (Fig. 6). Furthermore, a table in the Online-Resource 2 lists a

direct comparison of cpSSR haplotype vs. structure-based proportion at tree level for each population. However, we found no putative F1 hybrids. Because of the low differentiation between Slavonian and indigenous oaks at nSSRs, the identification of F1 hybrids is challenging. While adult stand and natural regeneration of stand IND_160 B are clearly assigned to the "indigenous" cluster, seedlings and adult trees of IND_159B show a variable assignment which is also reflected in the PCoA analysis and cpSSR haplotype composition (see above, Fig. 3).

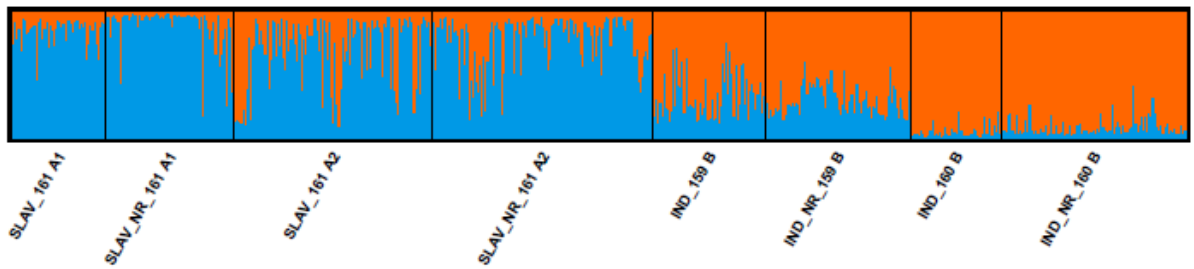


Figure 5 STRUCTURE diagram with admixed model and LOCPRIOR for K=2 displayed using CLUMPAK (Kopelman et al. 2015). Populations separated by black vertical lines. Blue and SLAV=Slavonian oak, orange=common oak for all populations, NR=natural regeneration, SLAV=Slavonian stand, IND=indigenous stand.

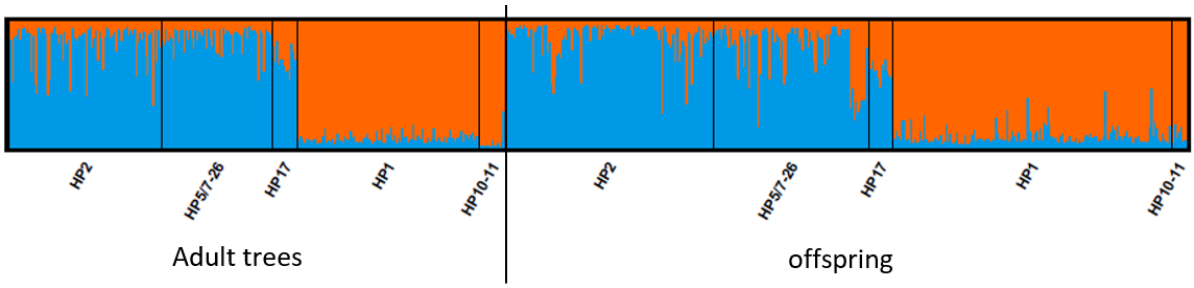


Figure 6 Differentiation of haplotypes based on nSSRs (admixed model with LOCPRIOR) displayed using CLUMPAK (Kopelman et al. 2015). Populations separated by black vertical lines. More orange=indigenous haplotype. blue=Slavonian haplotype.

**Table 5** STRUCTURE proportion of cluster 1 and cluster 2. NR=natural regeneration, SLAV=Slavonian stand, MIX=mixed stand, IND=indigenous stand.

| **Variety** | Indigenous | Slavonian |
|---|---|---|
| **Colour** | Orange | Blue |
| **Population** | **Proportion of cluster 1** | **Proportion of cluster 2** |
| SLAV_161 A1 | 0.139 | 0.861 |
| SLAV_NR_161 A1 | 0.096 | 0.904 |
| SLAV_161 A2 | 0.300 | 0.700 |
| SLAV_NR_ 161 A2 | 0.214 | 0.786 |
| IND_159 B | 0.655 | 0.345 |
| IND_NR_159 B | 0.657 | 0.343 |
| IND_160 B | 0.942 | 0.058 |
| IND_NR_160 B | 0.907 | 0.093 |

To show the genetic relationship between the 8 studied populations (4 adult stands and their natural regeneration), the UPGMA dendrogram was also prepared in the appendix, based on the unbiased genetic distance matrix of of Nei (1972) (Fig. S. 4). In accordance with PCoA (Fig. 3), it shows that the Slavonian and native stands differ only slightly at nuclear markers.

## 4.4 Discussion

### 4.4.1 Genetic diversity of adults and natural regeneration

To our knowledge, nuclear microsatellites are among the most precise tools for genotype determination and are important genetic markers widely used in population genetic analysis in oaks. According to Spence et al. (2021), microsatellites are an accessible method to gain an understanding of genetic diversity and structure.

A previous study (Burger et al. 2021) conducted on a larger number of populations enables the assessment of genetic differentiation between native and Slavonian adult stands using the same nuclear microsatellite marker set (except for 3 additional markers used in the present study). Accordingly, our dataset also shows high and similar genetic diversity at the microsatellite loci examined (Table 4 and Online Resource 1) across all populations with expected heterozygosity ($H_e$) of 0.545 and allelic richness ($A_R$) of 6.23. Such a relatively high genetic variation is common for outcrossing and wind pollinated woody species such as oaks (e.g., Shi et al. 2017: $H_e$=0.707, $A_R$=7.79; Spence et al. 2021: $H_e$=0.73, $A_R$=5.29). Furthermore, the overall pattern of genetic variation we observed at nuclear microsatellite loci is typical of long-lived and outcrossing species such as trees with relatively high genetic diversity ($A_R$=6.23, $H_e$=0.545), a low level of inbreeding within ($F_{IS}$=0.059) and a low

differentiation among populations ($F_{ST}$=0.022) (Hamrick et al. 1992). According to Kremer and Petit (1993) such a low species differentiation is typically for European white oaks.

No clear differences between the genetic variation indices of the natural regeneration and the adult trees were found in the stands of Hamm-Westtünnen in North-Rhine Westphalia (Table 4). Other studies, however, showed higher genetic diversity estimates, but also no clear differences between adult and progeny of pedunculate oak stands. For instance, based on 10 genomic microsatellite loci, Vranckx et al. (2014) reported for the adult trees a mean number of $A_R$ of 11.5, a mean $H_o$ of 0.798, a mean $H_e$ of 0.821, and for the progeny a mean $A_R$ of 10.7, a mean $H_o$ of 0.804, and a mean $H_e$ of 0.817. Based on 19 nuclear microsatellite markers (12 genomic and 7 EST-SSRs), also Sandurska et al. (2017) reported slightly higher values (adult: $A_R$=17.823, $H_o$=0.784, $H_e$=0.821; seedlings: $A_R$=17.128, $H_o$=0.759, $H_e$=0.803) as compared to the present study. However, in assessing diversity indices from different studies, it should be mentioned that the values are not directly comparable, as different microsatellite marker sets have been used by different researchers. For instance, higher genetic variation could be due to the generally higher variability at genomic SSRs compared with EST-SSRs (Rungis et al. 2004).

The estimates of $F_{IS}$ were positive and show significant deviations from HWE at some loci (Online-Resource 1). Positive $F_{IS}$ values indicate inbreeding only if they are significant at most loci, which was not the case here (only at 2 loci per population, Online-Resource 1). Otherwise, high $F_{IS}$ values at only certain loci may indicate selection for these loci, selection for genes associated with these loci, or the occurrence of null alleles (Bogdan et al. 2018). However, due to the very low null allele frequency (0.51 % to 2.71%) on average across loci, little impact on population genetic structures and differentiation ($F_{ST}$) can be assumed based on Oddou-Muratorio et al. (2009) and Chapuis und Estoup (2007).

Positive and higher $F_{IS}$ in the Slavonian adult and offspring cohorts as compared to the indigenous stands could be due to the Wahlund effect and non-random mating (e.g., inbreeding, or preferential mating within groups of different geographic origin, differences in flowering time). However, no clear effects of genetic drift and inbreeding on the genetic diversity of the progeny could be observed, so non-random mating is rather unlikely.

Reproductive isolation of late flushing Slavonian oaks from indigenous stands has likely resulted in the observed pattern of genetic differentiation between Slavonian and indigenous seedling cohorts. Here, it is important to note that the flushing date was assessed a proxy for flowering time, two traits which are highly correlated in oaks (e.g., Chesnoiu et al. 2009). Low coefficients of pairwise fixation levels show also low difference between the two generations ($F_{ST}$=0.013) of the studied stands, thus providing no evidence for fixation or genetic drift. In addition, the overall effect of genetic drift

on allelic richness ($A_R$) was also rather limited, and even when all populations were pooled, allelic richness was not significantly different between adult and seedling cohorts (Table 4).

Likewise, the analysis of molecular variance for the two generations shows greater variability within populations (98 %) than among populations (2 %), which is commonly observed in pedunculate oaks (Gömöry et al. 2001, Neophytou 2015, Burger et al. 2021, Kesić et al. 2021). Furthermore, a comparison between Slavonian ($A_R$=6.26, $H_o$=0.504, $H_e$=0.542, $F_{IS}$=0.077) and indigenous ($A_R$=6.13, $H_o$=0.523, $H_e$=0.548, $F_{IS}$=0.055) old-growth stands and their natural regeneration (SLAV_NR: $A_R$=6.10, $H_o$=0.521, $H_e$=0.550, $F_{IS}$=0.055; IND_NR: $A_R$=5.54, $H_o$=0.528, $H_e$=0.541, $F_{IS}$=0.030) also revealed similar levels of genetic variation. In addition, similar diversity values of the introduced plantations as compared to native stands, suggest that Slavonian stands were established with seed material that was sampled in Croatia from a representative number of trees per population.

The low genetic differentiation between generation and similar levels of expected heterozygosity and allelic richness in the seedlings of Slavonian stands indicated that natural regeneration is efficient in these stands, possibly due to the low density of herbivores/deer. According to Hamrick (2004), sustaining high genetic diversity increases the likelihood of having well-adapted genotypes to ensure the long-term viability of forest tree populations.

### 4.4.2 Haplotype composition of adult stands and their natural regeneration

Since chloroplast markers (cpSSRs) in angiosperms are usually maternally inherited (Dumolin et al. 1995) and natural regeneration share the same chloroplast information as the maternal tree, they show high uniformity within stands but marked differentiation among them, which is especially true for barochoric tree species such as oaks (Petit et al. 2005). Thus, chloroplast markers are particularly suitable in combination with biparentally inherited microsatellite markers to classify and detect admixture of natural regeneration of Slavonian and indigenous oaks. The five cpSSRs used in this study have been shown to be suitable and useful in previous studies to identify different haplotypes (Gailing et al. 2007a, 2007b, 2009).

The populations analyzed in this study were first examined for haplotype composition in 2003 by Gailing et al. (2003) on 10 representative individuals per population. Thereby, haplotypes 1-4 found in Gailing et al. (2003), correspond to haplotypes 2, 5, 10 and 1 described in Petit et al. (2002a). We found similar haplotype composition for the SLAV_161 A1, SLAV_161 A2, IND_159 B, and IND_160 B stands as described in Gailing et al. (2003), except that HP1 and HP17 were additionally found in individual trees in SLAV_161 A2 and HP2, HP5/7-26, and HP17 in IND_159 B (Table 3, Fig. 2). Since haplotype 5/7-26 also occurs naturally in Germany, it is not possible to infer Slavonian reproductive material from cpSSR markers alone. Therefore, the combination of both marker types (maternally

inherited cpSSRs and biparental inherited nSSRs) was useful to detect this admixture and to assign haplotypes, which occur naturally in both Germany and Slavonia, to their geographic origin.

The natural regeneration shows a similar to equal haplotype composition compared to the respective adult stands (Table 3). This can be also seen in the PCoA based on cpSSRs (Fig. 4), which assigns each natural regeneration to its respective old-growth stand with low genetic distances. Only the relative frequencies of haplotypes differ slightly between adults and seedlings (Fig. 2), but the most frequent haplotype remains the same in both generations (Table 3).

### 4.4.3 Genetic assignment of populations

We used the Bayesian model-based clustering method implemented in the program STRUCTURE (version 2.3.4, Pritchard et al. 2000) to assign individuals to K populations based on 23 nuclear microsatellites. The STRUCTURE analysis (Fig. 5, Table 5) revealed that the studied populations were grouped into two clusters, Slavonian (more proportion of blue) and indigenous oaks (more proportion of orange). However, it is difficult to identify two distinct groups (Slavonian and Native) based on the PCoA (Fig. 3). Since the natural regeneration of each adult stand is shifted substantially against their maternal stands in one direction. On the one hand, a possible reason for the shift in natural regeneration IND_NR_159 B and IND_NR_160 B along axis 1 could be gene flow between native stands (e.g., the surrounding native stands that were not sampled, cf. Fig. 1). On the other hand, the shifts in the natural regeneration of the Slavonian stands could suggest a (non-random) gene flow (pollen influx) from another Slavonian stand (Table S. 4, 5, Fig. S. 4). However, a PCoA with all Slavonian and native populations in North Rhine-Westphalia from Burger et al. (2021) and the populations investigated in this study also revealed two groups in which the Slavonian populations (SLAV) could be assigned to the Slavonian cluster of other Slavonian populations in North Rhine-Westphalia (Fig. S. 5). Therefore, the nuclear marker set is well suited to distinguish at the population level (but not at the tree level) between Slavonian and native oak in both old-growth stands and their progeny and to identify mixed stands (see stand IND_159 B). In addition to these nuclear marker results, the inclusion of cpDNA haplotypes shows that Slavonian and indigenous oak stands are not always pure stands (Fig. 2). However, the Slavonian stand SLAV_161 A2 also showed 18 % of the non-Slavonian haplotypes 1 and 10-11, and IND_159 B showed 25 % Slavonian old-growth trees (HP5/7-26, 5, 17) (Fig. 2). We demonstrated that chloroplast markers are suitable to identify individual trees (tree level) with non-Slavonian haplotypes in Slavonian old-growth stands and vice versa.

Comparison of STRUCTURE results with cpSSR haplotypes for haplotype 2 showed that within the parent population 85 trees had HP2 and of these 73 were also assigned to the Slavonian cluster

(ancestry coefficient >0.6) based on nSSRs (Online-Resource 2, Fig. 6). Thus, 86% of the parent trees with haplotype 2 were also assigned to the Slavonian variety based on nSSRs.

Furthermore, low genetic differentiation between Slavonian and indigenous oaks did not allow to unambiguously distinguish between varieties at the tree level (Online-Resource 2). The different timing of bud burst (Table 1), as described for these stands in Gailing et al. (2003) suggests that flowering times between taxa show little to no overlap (due to synchronization of flowering with flushing, Chesnoiu et al. 2009), thus restricting gene flow between Slavonian and indigenous stands. In Germany, Slavonian oaks show up to three weeks later bud burst compared to indigenous oak stands (Wachter 2001, Gailing et al. 2003).

### 4.4.4 Practical applicability

For sustainable forest management, it is important to provide forest enterprises with reproductive material that complies with both economic objectives, such as increment and wood quality, and ecological objectives, such as genetic diversity and local adaptation (Konrad et al. 2012). In this context, Slavonian oak is currently gaining interest as a seed source for forestry not only because of its very good economic characteristics (wood quality, fast height growth, long clear stem), but also with regard to climate change-induced forest conversion due to its broad site amplitude, possibly better adaption and late bud burst and associated lower susceptibility to the oak leaf roller (Schirmer 2017, Rieger 2018, Burger and Gailing 2021). Currently, an alternative form of sustainable forest management is practiced, based on certification systems such as certification of the origin of reproductive material, which provides the forest owner with detailed information about the origin of the planting material. The microsatellite marker set (cpSSRs and nSSRs) used in this study can reliably be used for identification and certification of Slavonian and native forest reproductive material. For this purpose, a previous study (Burger et al. 2021) already indicated that this nuclear microsatellite marker set is suitable for identifying mixed stands, detecting the origin of reproductive material, and distinguishing between Slavonian and native oak stands.

Furthermore, the marker set is also suitable for establishment and test of admixture in seed orchards. Thus, Bogdan et al. (2018) based on other nSSRs and the same cpSSRs were able to investigate clonal seed orchards in Croatia for their genetic diversity and differentiation as well as spatial genetic structure and transfer of forest reproductive material between clonal seed orchards.

Meanwhile, there are also some late flushing Slavonian stands in Germany for seed production and breeding, such as the selected provenances (“Sonderherkünfte”) "Münsterländer Späteiche – Slawonische Stieleiche", "Königsforst" as well as "Kottenforst" from North Rhine-Westphalia and the "Späteiche Burg Eltz" from Rhineland-Palatinate (DKV 2022).

In a further study, however, it would be necessary to investigate the extent to which gene flow occurs between intermediate and early flushing stands. Especially seed stands must be chosen to avoid outside pollen influx. The advantage in this case could be that plantations or seed orchard with the particularly late-flushing haplotype 2 may not require geographic isolation from conspecific indigenous stands, as they are reproductively isolated from early-flushing neighboring stands due to different flowering times.

## 4.5 Conclusion and future perspectives

Our study shows that the genetic information of old-growth stands is almost completely passed on to the next generation. Minor changes in the genetic structure from adult to offspring generation suggest the maintenance of the same level of genetic variation in the progeny. Since genetic diversity has a strong impact on the adaptability of populations to changing environmental conditions and survival, the main objective for effective conservation of forest genetic resources is to achieve the highest possible level of genetic variability (Andonovski and Velkovski 2019). In addition, the genetic variability of tree populations ensures the stability and sustainability of forest ecosystems. Moreover, limited gene flow was revealed between the native and Slavonian oaks, likely due to differences in bud burst and flowering time between the two oak taxa, especially between the very late-flushing (predominantly haplotype 2) and early-flushing (predominantly haplotype 1) oaks.

In conclusion, seed production stands and seed orchards of late flushing Slavonian oak would require little geographic isolation from early flushing indigenous oak stands.

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Kapitel 4

# Supplementary Material

**Table S. 1** Chloroplast microsatellites (cpSSRs)

| Locus | Primer sequences (5'-3') | Motif | Size (bp) | $T_a$ (°C) | Location | Reference |
|---|---|---|---|---|---|---|
| *udt 1* | HEX-ATCTTACACTAAGCTCGGAA<br>TTCAATAACTTGTTGATCCC | $(A)_{11}$ | 80–82 | touch down | *trnE-trnT* Intergenic | Deguilloux et al. (2003) |
| *ucd 4* | FAM-TTATTTGTTTTTGGTTTCACC<br>TTTCCCATAGAGAGTCTGTAT | $(T)_{12}$ | 93–96 | touch down | *ycf6-psbM* Intergenic | Deguilloux et al. (2003) |
| *udt 4* | FAM-GATAATATAAAGAGTCAAAT<br>CCGAAAGGTCCTATACCTCG | $(A)_9$ | 143–165 | touch down | *trnE-trnT* Intergenic | Deguilloux et al. (2003) |
| *ccmp2* | FAM-GATCCCGGACGTAATCCTG<br>ATCGTACCGAGGGGTTCGAAT | $(A)_{11}$ | 233–234 | 53 °C | *5' to trnS* | Weising and Gardner (1999) |
| *ccmp10* | HEX-TTTTTTTTTAGTGAACGTGTCA<br>TTCGTCGDCGTAGTAAAATAG | $(T)_{14}$ | 111–112 | 53 °C | *rpl2-rps19* intergenic | Weising and Gardner (1999) |

**Table S. 2** Haplotype identification with cpSSRs based on Gailing et al. (2007a, 2007b, 2009). Size in base pairs (bp).

| Haplotype | *udt1* | *ucd4* | *udt4* | *ccmp2* | *ccmp10* |
|---|---|---|---|---|---|
| **HP1** | 80 | 95 | 145 | 233 | 112 |
| **HP2** | 80 | 93 | 145 | 233 | 111 |
| **HP5** | 80 | 94 | 144 | 233 | 112 |
| **HP7-26** | 80 | 94 | 144 | 233 | 112 |
| **HP10-11** | 81 | 95 | 143 | – | – |
| **HP17** | 81 | 96 | 165 | 234 | 111 |

Table S. 3 Summary of the genetic parameters and null alleles per loci. agSSRs developed from Q. rubra. bEST-SSRs developed for Q. rubra. without sign=EST-SSRs developed for *Q. robur* and *Q. petraea*.

| Mean across all populations | OC11[a] | 2P24[a] | 3A05[a] | 3D15[a] | FIR013 | FIR028 | FIR035 | FIR043 | GOT040 | PIE040 | PIE125 | VIT023 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **N** | 71.100 | 71.900 | 71.600 | 71.300 | 71.800 | 70.500 | 71.500 | 70.700 | 71.100 | 70.600 | 71.200 | 71.100 |
| **$H_o$** | 0.795 | 0.092 | 0.102 | 0.387 | 0.689 | 0.632 | 0.627 | 0.752 | 0.818 | 0.423 | 0.665 | 0.134 |
| **$H_e$** | 0.879 | 0.088 | 0.106 | 0.418 | 0.672 | 0.789 | 0.658 | 0.823 | 0.829 | 0.579 | 0.691 | 0.134 |
| **$F_{ST}$** | 0.017 | 0.009 | 0.010 | 0.014 | 0.017 | 0.027 | 0.016 | 0.019 | 0.013 | 0.023 | 0.017 | 0.016 |
| **G'stH** | 0.092 | 0.002 | 0.003 | 0.011 | 0.033 | 0.096 | 0.027 | 0.069 | 0.035 | 0.035 | 0.034 | 0.010 |
| **Frequency of null alleles** | 0.036 | – | – | 0.010 | – | 0.081 | 0.019 | 0.023 | 0.006 | 0.092 | 0.006 | – |
| **Frequency of null alleles across loci** | | | | 0.012 | | | | | | | | |

| Mean across all populations | VIT107 | FIR104 | Pie102 | Pie267 | Qr0332[b] | Qr0057[b] | Qr1423[b] | FS_C2361[b] | FS_C2660[b] | FS_C2791[b] | FS_C8183[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **N** | 70.200 | 71.600 | 71.700 | 71.700 | 71.700 | 71.700 | 71.600 | 71.800 | 71.100 | 71.200 | 71.500 |
| **$H_o$** | 0.792 | 0.689 | 0.562 | 0.797 | 0.603 | 0.472 | 0.171 | 0.500 | 0.629 | 0.254 | 0.295 |
| **$H_e$** | 0.872 | 0.686 | 0.592 | 0.793 | 0.597 | 0.455 | 0.167 | 0.483 | 0.644 | 0.274 | 0.282 |
| **$F_{ST}$** | 0.029 | 0.011 | 0.020 | 0.016 | 0.174 | -0.016 | 0.013 | 0.012 | 0.021 | 0.010 | 0.022 |
| **G'stH** | 0.191 | 0.013 | 0.033 | 0.047 | 0.452 | 0.024 | 0.007 | 0.010 | 0.043 | 0.002 | 0.F022 |
| **Frequency of null alleles** | 0.024 | – | – | – | – | 0.013 | – | – | 0.013 | 0.016 | - |
| **Frequency of null alleles across loci** | | | | 0.021 | | | | | | | 0.006 |

**Table S. 4** Mean pairwise $F_{ST}$ and $G'_{ST}$(Hed) between A) all adult trees, all adult and progeny as well as between all progenies and B) indigenous oaks, Slavonian and indigenous oaks and between Slavonian oaks.

| A) | Pairwise | | B) | Pairwise | |
|---|---|---|---|---|---|
| Between the group of | $F_{ST}$ | $G'_{ST}$(Hed) | Between the group of | $F_{ST}$ | $G'_{ST}$(Hed) |
| **All adults** | 0.010 | 0.022 | **Indigenous oaks** | 0.010 | 0.027 |
| **All adults and progenies** | 0.013 | 0.038 | **Slavonian and indigenous** | 0.014 | 0.042 |
| **All progenies** | 0.014 | 0.046 | **Slavonian oaks** | 0.009 | 0.022 |

**Table S. 5** Mean pairwise $F_{ST}$ and $G'_{ST}$(Hed) between adult trees, adult trees and progeny as well as between progenies separated by Slavonian and indigenous populations.

| | Slavonian populations | | Indigenous populations | |
|---|---|---|---|---|
| | Pairwise | | Pairwise | |
| Between the group of | $F_{ST}$ | $G'_{ST}$(Hed) | $F_{ST}$ | $G'_{ST}$(Hed) |
| **Adult** | 0.005 | 0.003 | 0.010 | 0.021 |
| **Adult and progeny** | 0.012 | 0.036 | 0.009 | 0.023 |
| **Progeny** | 0.014 | 0.046 | 0.013 | 0.039 |

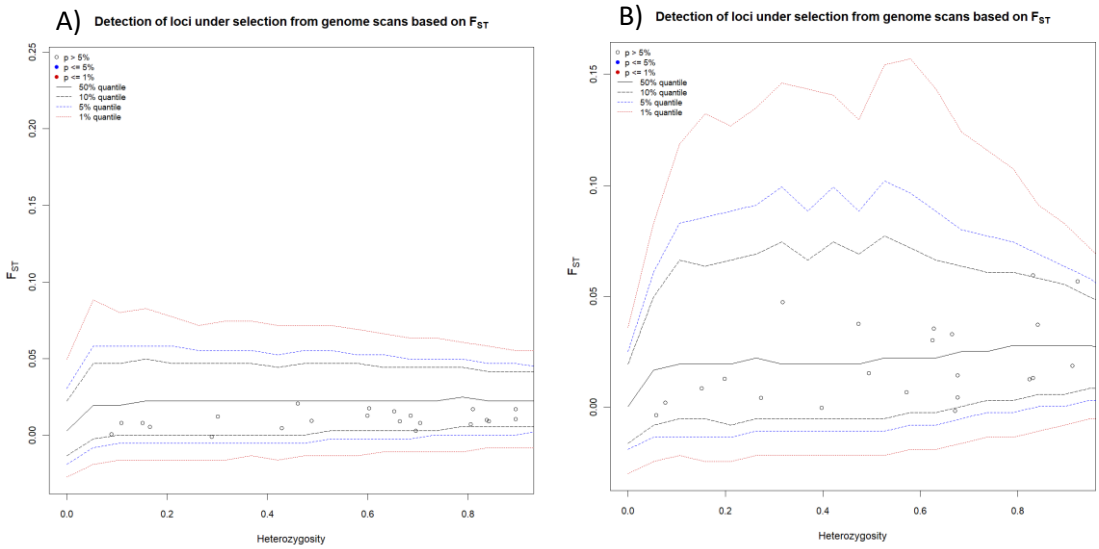



**Figure S. 1** Outlier analysis of 23 markers A) between all populations and B) between clearly indigenous (IND_160_B) and Slavonian (SLAV_161 A1) stands.

Table S. 6 Presence of null alleles.

| Population | Locus | Frequency of null alleles | Across loci |
|---|---|---|---|
| SLAV_161 A2 | OC11 | 0.0857 | 1.5% |
| | PIE040 | 0.114 | |
| | Pie125 | 0.0545 | |
| | VIT107 | 0.0966 | |
| SLAV_161 A1 | FIR028 | 0.0925 | 2.0% |
| | FIR043 | 0.0765 | |
| | OC11 | 0.0824 | |
| | VIT107 | 0.0506 | |
| | FS_C2791 | 0.1615 | |
| IND_160 B | FIR028 | 0.1162 | 0.51% |
| IND_159 B | PIE040 | 0.1716 | 1.87% |
| | FS_C2660 | 0.1315 | |
| | Qr0057 | 0.1269 | |
| MIX_159 A | 3D15 | 0.1049 | 1.75% |
| | FIR028 | 0.1141 | |
| | OC11 | 0.0634 | |
| | PIE040 | 0.1204 | |
| SLAV_NR_161 A2 | FIR028 | 0.07 | 1.62% |
| | FIR043 | 0.0602 | |
| | OC11 | 0.0603 | |
| | PIE040 | 0.1464 | |
| | VIT107 | 0.0349 | |
| SLAV_NR_161 A1 | FIR028 | 0.1726 | 2.51% |
| | FIR035 | 0.0987 | |
| | OC11 | 0.0661 | |
| | GOT040 | 0.0554 | |
| | PIE040 | 0.1848 | |
| IND_NR_160 B | FIR028 | 0.123 | 0.53% |
| IND_NR_159 B | FIR028 | 0.1258 | 2.39% |
| | FIR035 | 0.0877 | |
| | FIR043 | 0.0976 | |
| | PIE040 | 0.1858 | |
| | VIT107 | 0.0525 | |
| MIX_NR_159 A | FIR028 | 0.1155 | 1.6 % |
| | PIE040 | 0.1386 | |
| | VIT107 | 0.1133 | |

**Table S. 7** Bottleneck results based on Wilcoxon's signed-rank test (one-tailed=W_1t). No values are significant at α=0.05. N=population size; k=number of alleles; $H_e$=heterozygosity; TPM=two phase-mutation model; SSM=stepwise mutation model. NR=natural regeneration, SLAV=Slavonian stand, MIX=mixed stand, IND=indigenous stand.

| Population | Mean_N | Mean_k | Mean_$H_e$ | p_W_1t_TPM | p_W_1t_SMM | Allele frequency distribution |
|---|---|---|---|---|---|---|
| **SLAV_161 A2** | 214.00 | 1.22 | 0.02310 | 0.984375 | 0.984375 | L-shaped |
| **SLAV_161 A1** | 101.57 | 1.26 | 0.02391 | 0.984375 | 0.992188 | L-shaped |
| **IND_160 B** | 99.22 | 1.22 | 0.01106 | 1.0 | 1.0 | L-shaped |
| **IND_159 B** | 121.30 | 1.22 | 0.02033 | 0.984375 | 0.984375 | L-shaped |
| **MIX_159 A** | 77.39 | 1.22 | 0.01951 | 0.984375 | 0.984375 | L-shaped |
| **SLAV_NR_161 A2** | 237.74 | 1.30 | 0.01960 | 0.996094 | 0.996094 | L-shaped |
| **SLAV_NR_161 A21** | 139.48 | 1.26 | 0.02453 | 0.976563 | 0.984375 | L-shaped |
| **IND_NR_160 B** | 197.39 | 1.22 | 0.01068 | 1.0 | 1.0 | L-shaped |
| **IND_NR_159 B** | 156.87 | 1.26 | 0.01749 | 1.0 | 1.0 | L-shaped |
| **MIX_NR_159 A** | 140.87 | 1.30 | 0.02147 | 1.0 | 1.0 | L-shaped |

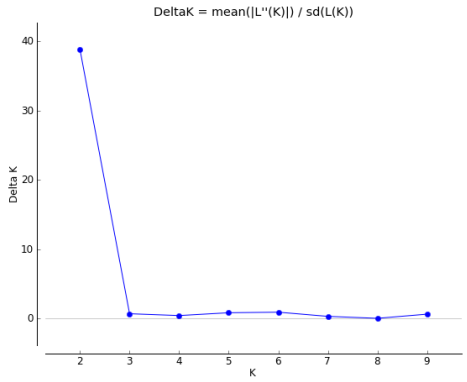



**Figure S. 2** STRUCTURE HARVESTER results to determine the most likely K value according to the Evanno et al. (2005) method. which compares the ΔK between sequential K values. Function graph for determining the optimal K.

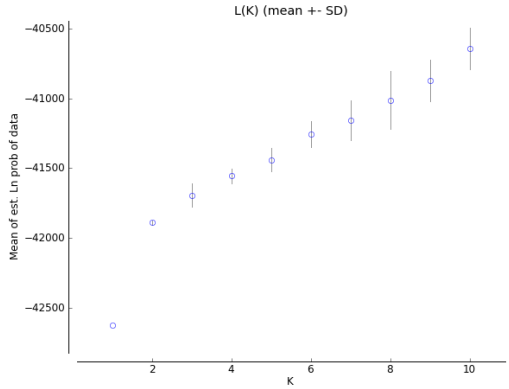



**Figure S. 3** Comparison of the mean of the estimate of the natural log of the probability of the data amongst K values. with the circle centered over the mean and the bar indicating the standard error.

**Table S. 8** Results of the Evanno method for the best K.

| K | Reps | Mean LnP(K) | Stdev LnP(K) | Ln'(K) | \|Ln''(K)\| | Delta K |
|---|---|---|---|---|---|---|
| 1 | 9 | -42622.455556 | 0.194365 | — | — | — |
| 2 | 10 | -41888.550000 | 13.878541 | 733.905556 | 538.825556 | 38.824367 |
| 3 | 10 | -41693.470000 | 83.127854 | 195.080000 | 57.190000 | 0.687976 |
| 4 | 10 | -41555.580000 | 54.674304 | 137.890000 | 23.240000 | 0.425063 |
| 5 | 10 | -41440.930000 | 84.161789 | 114.650000 | 69.960000 | 0.831256 |
| 6 | 10 | -41256.320000 | 92.666173 | 184.610000 | 84.440000 | 0.911228 |
| 7 | 10 | -41156.150000 | 140.447865 | 100.170000 | 43.330000 | 0.308513 |
| 8 | 10 | -41012.650000 | 206.140342 | 143.500000 | 5.510000 | 0.026729 |
| 9 | 10 | -40874.660000 | 147.507116 | 137.990000 | 92.380000 | 0.626275 |
| 10 | 10 | -40644.290000 | 146.679435 | 230.370000 | — | — |

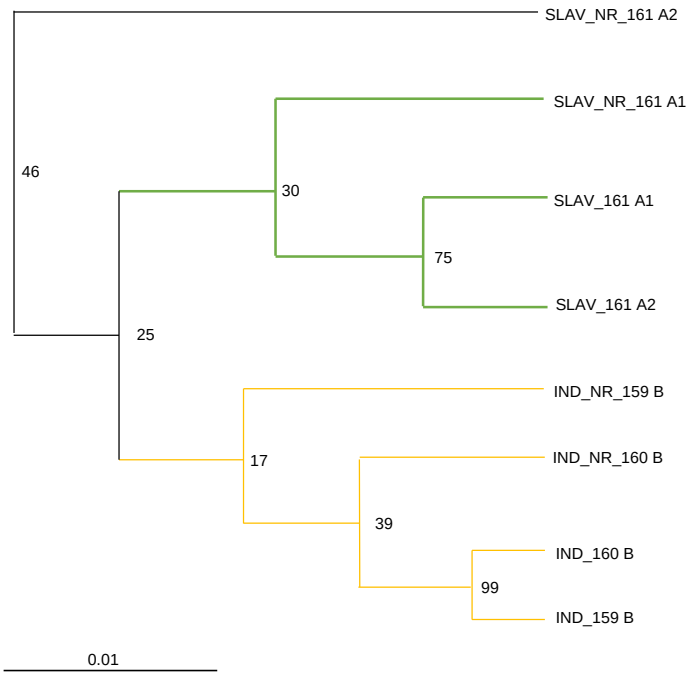



**Figure S. 4** UPGMA dendogram based on Nei's standard genetic distance DS (Nei 1972) with bootstrap values based on 1000 permutations across loci. Green=group of Slavonian oaks, yellow=group of indigenous oaks. NR=natural regeneration, SLAV=Slavonian stand, IND=indigenous stand.

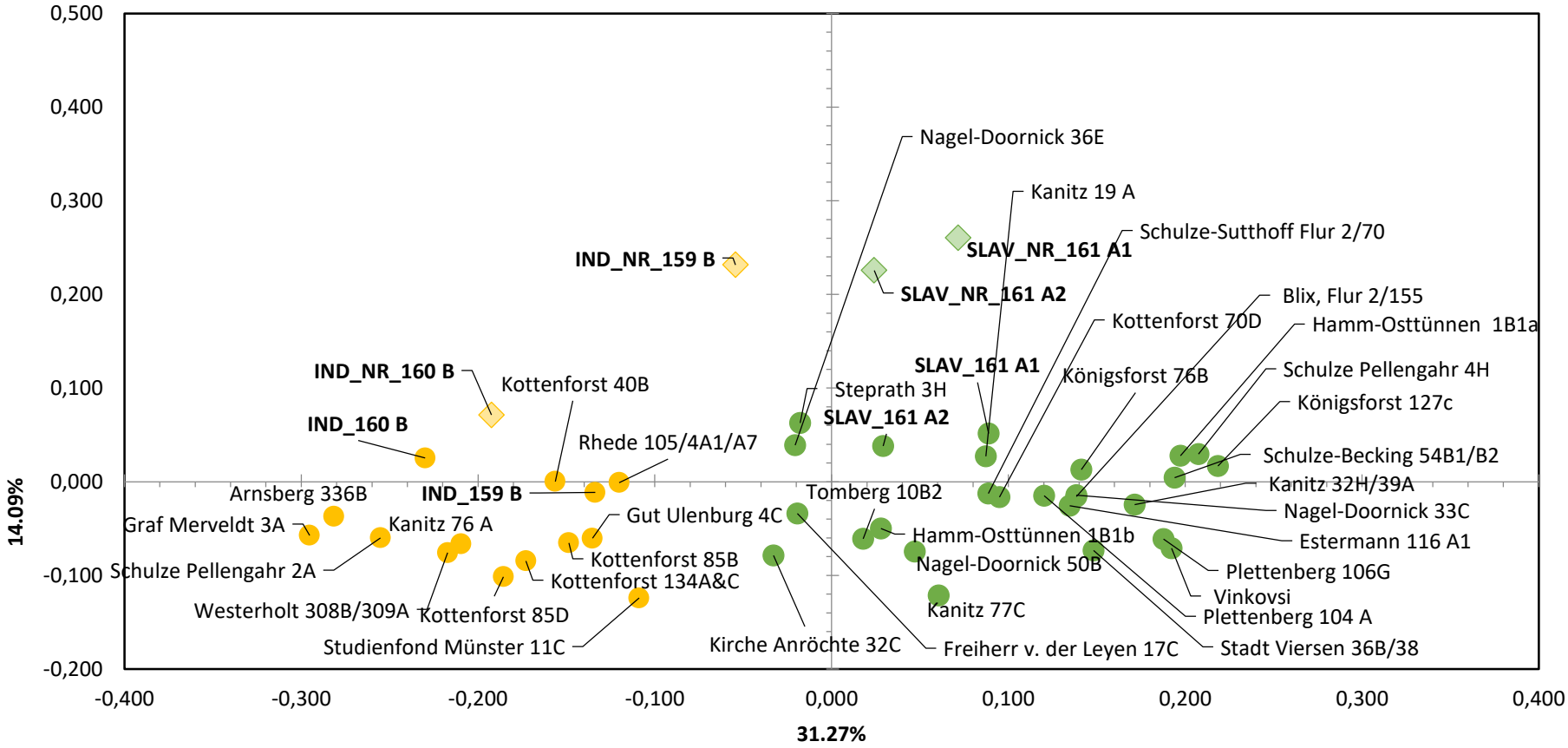



**Figure S. 5** Principal coordinate analysis (PCoA) based on nuclear microsatellite markers (nSSRs, except marker OC11, FIR041 and PIE040) for all populations from Burger et al. (2021) and the current study (marked bold). Colour green and SLAV=Slavonian stand, yellow and IND=indigenous stand, circle=old stands, square=natural regeneration.

# Kapitel 5

## RADseq based high-density genetic mapping in a *Quercus robur* full-sib family (*Quercus robur* L. x *Quercus robur* L. subsp. *slavonica* (Gáyer) Mátyás)

This chapter has not been part of a peer-reviewed publication to date, but is left open for publication in the future. Katrin Schmidt established the concept and wrote the text. Oliver Gailing improved the manuscript with valuable comments.

# RADseq based high-density genetic mapping in a *Quercus robur* full-sib family (*Quercus robur* L. x *Quercus robur* L. subsp. *slavonica* (Gáyer) Mátyás)

## Abstract

Given the pressing global climate change challenges, there is a significant demand for a comprehensive understanding of trait selection in tree species to facilitate informed forest management. In this study we constructed genetic linkage maps for both parents of a *Quercus robur* x *Quercus robur* subsp. *slavonica* full-sib pedigree based on restriction site-associated DNA sequencing (RADseq). In the present study, 249 progenies of a controlled intraspecific cross of pedunculate oak were used to generate genetic linkage maps. The construction of the genetic linkage maps was done using the *pseudo-testcross* method and *Kosambi's* mapping function. The number of linkage groups (12) corresponds to the number of chromosome pairs (n=12) of the mapped progeny. A total of 473 SNP markers and 502 SNP markers were mapped on the female and male linkage maps, respectively. The total map length and average marker spacing were 885 cM and 1.87 cM for the female as well as 898.4 cM and 1.79 cM for the male map. Genetic linkage maps based on RADseq provide a valuable foundation for conducting future genetic analyses such as QTL analysis, identification of genes involved in adaptive trait variation and comparative mapping.

**Key words**: *Quercus robur*, genetic linkage maps, full-sib family, Slavonian oak, high-density genetic mapping

## 5.1 Introduction

Oaks (genus *Quercus*, family Fagaceae) are among the most common and widespread tree species and represent an ecologically as well as economically important resource in subtropical, Mediterranean, and temperate areas of the Northern Hemisphere (Aldrich and Cavender-Bares 2011, Barreneche et al. 1998, Curtu et al. 2007, Dumolin et al. 1995). Within the genus *Quercus*, pedunculate oak (*Quercus robur* L.) is predominant in Germany and distributed almost all of Europe, and thus one of the most important deciduous tree species (Barreneche et al. 1998, Reif et al. 2016). Because of their wide geographic distribution as dominant tree species and the diverse climatic and edaphic conditions of growing areas, oaks can be regarded as model tree species for the study of adaptation to different environments as well as differentiation at the genome level. Thereby, oaks have repeatedly been the focus of genetic, evolutionary and phylogeographic studies in the last decades (Aldrich and Cavender-Bares 2011; Kremer et al. 2007). *Quercus robur* is a diploid outcrossing species (2n=2x=24) with a genome size of 1.5 Gbp/2C (Plomion et al. 2016). Compared to data available on other woody angiosperms (e.g. *Populus trichocarpa* (Torr. & Gray) ca. 500 Mbp (Tuskan et al. 2006), *Fagus sylvatica* L. 541 Mbp (Mishra et al. 2022), *Eucalyptus grandis* W. Hill 640 Mbp (Grattapaglia and Bradshaw Jr., 1994), *Fraxinus excelsior* L. ca. 877 Mbp (Sollars et al. 2017)) the physical genome size of *Q. robur* is larger, however its genome size is among the lower values found in other angiosperms (e.g. *Ranunculus ficaria* 1C/9.12 Gbp; *Hyacinthoides non-scripta* 1C/20.73 Gb; *Fritillaria meleagris* 1C/46.26 Gbp; *Paris japonica* 1C/148.8 Gbp) (Pellicer et al. 2018).

The genetic linkage map is a fundamental and powerful tool for genetics research. A genetic linkage map consists of a number of molecular markers arranged on so-called linkage groups. The number of linkage groups corresponds ideally to the number of chromosome pairs of the mapped species. It provides a basis for comparative genomics and reveals important insights for understanding genome evolution and divergence (Kai et al. 2014, Kawakami et al. 2014, Liu et al. 2016). Genetic linkage maps play an important role in many areas in the field of genetics, such as plant breeding, heredity, genome assembly and evolution, candidate gene identification, localization of chromosomal regions associated with certain (phenotypic) traits of the mapped species, quantitative trait loci (QTLs), marker-assisted selection as well as map based cloning of genes (Cheema and Dicks 2009, Gailing 2008, Gailing et al. 2013, Jansen 1993, Liu et al. 2016, Wu et al. 2008). Moreover, genetic linkage maps create connections and facilitate the mapping of the association of phenotypes and genotypes (QTL mapping), thus enabling the research of the genetic basis of complex phenotypic traits (Wang et al. 2011).

Previous genetic linkage maps in different intraspecific crosses of *Q. robur* have been based on microsatellites (SSRs, EST-SSRs), Amplified Fragment Length Polymorphism (AFLPs), RAPDs, isozymes,

and 5S rDNA markers (Barreneche et al. 1998, Gailing 2008, Gailing et al. 2013, Saintagne et al. 2004) as well as SNP markers (SNPs generated from resequencing and the oak Unigene) (Bodénès et al. 2016). However, these markers, such as AFLPs, RAPDs and microsatellites, used to construct such maps, are all challenging because they are either difficult to develop or anonymous (no functional meaning, not corresponding to a specific gene) and difficult to convert into useful sequence-based markers. Rapid developments in recent years in sequencing technologies, such as next generation sequencing (NGS), have expanded our ability to obtain whole or partial genomes in a relatively short time (Heather and Chain, 2016). Genotyping using high-throughput NGS technologies reduces the complexity of genomic analysis and enables the development of numerous SNP markers (Kumar et al. 2012). SNP markers are abundant and widely used in most species, with good genetic stability and high frequency. One of the preferred NGS-based sequencing technologies is restriction site-associated DNA sequencing (RADseq) and double-digested RADseq (ddRADseq) (Baird et al. 2008, Peterson et al. 2012). RADseq simplifies genetic variant discovery by sequencing only the DNA enclosing specific restriction enzyme sites, enabling detection of orthologous sequences in multiple individuals (Baxter et al. 2011). The RADseq method for SNP marker generation features ease of use, low cost compared to whole genome sequencing, high-throughput, and independence from genomic information, and are a practical approach for developing large numbers of genetic markers, creating a high-density linkage map, and studying the genetic architecture of complex traits in population genetics (Davey and Blaxter 2011, Peterson et al. 2012, Zhao et al. 2020).

The objective of our study was to construct the first high-density genetic linkage map within an intraspecific *Quercus robur* x *Q. robur* ssp. *slavonica* full-sib family based on SNP markers generated by the RADseq method.

## 5.2 Materials and Methods

### 5.2.1 Mapping population

A full-sib family originating from a controlled intraspecific cross of pedunculate oak [*Quercus robur* subsp*. robur* x *Quercus robur* subsp. *slavonica*] was generated in 2003 (Gailing 2008). The female parent is situated close to Escherode (Lower Saxony, latitude 51.19°N, longitude 9.40°E, altitude 380m) and shows haplotype 1, which is indigenous to Germany (König et al. 2002). The male parent was selected in Hüls (Hamm-Westtünnen, Münsterland, North Rhine-Westphalia, Abt. 161A1, owned by Freiherr v. Boeselager, altitude 36m) and is characterized by haplotype 5, late bud burst (up to three weeks later) and its phenology (high growth performance, straightness, a long clear bole) compared to indigenous oaks as well as its chloroplast haplotype HP5 (Gailing et al. 2003), which has a center of distribution in the Balkan region (Bordács et al. 2002).

According to historical documents and analysis with cpDNA and nSSRs markers, trees of the Slavonian stands in Münsterland have their geographic origin in the forest areas of the lowlands of the rivers Sava and Drava between Zagreb and Belgrade in the eastern region of Croatia (Burger et al. 2021, Burger and Gailing 2022, Gailing et al. 2007a, 2007b, Wachter 2001).

The controlled cross was carried out at the end of April 2003. Further details of the controlled crossing are described in Gailing (2008). The full-sib family was raised in the greenhouse of the Department of Forest Genetics and Forest Plant Breeding of the University of Göttingen from 2004 to 2006 and subsequently planted out in spring 2007 on a field near the university.

Plant material (buds) of in total 249 crossing progenies and 8 leave samples of both the maternal and paternal tree was collected in August 2019 (leaves) and January 2020 (buds).

## 5.2.2 DNA isolation, quantification and RAD sequencing

DNA was isolated from buds and leaves using the DNeasy® Plant Mini Kit from Qiagen (Hilden, Germany) following the standard Quick-Start Protocol (March 2016). Subsequently, the amount of DNA was checked for degradation on 1.5 % agarose gels. In addition, DNA concentration was determined fluorometrically using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific) on a Invitrogen™ Qubit™ 4 fluorometer (Thermo Fisher Scientific). DNA concentration was adjusted to 20 ng/µl (equivalent concentrations) across each sample in preparation for RADseq. In total, 264 DNA samples including 249 putative full-sibs and 8 DNA samples each from the seed and pollen parent were prepared for RAD-Seq. The normalized DNA samples were submitted to Floragenex Inc. (Beaverton OR, USA) for restriction site-associated DNA sequencing (RADseq) according to an established protocol described in Baird et al. (2008). Total genomic DNA was digested with the restriction endonuclease *PstI* and processed into multiplexed RAD libraries. Sequence analysis (sequencing chemistry 1x95 bp) of RADseq data was performed following the steps as described in Slavov et al. (2014). A total of 1,682,207,630 reads were obtained for all samples, with a mean of 6,207,408.2 and a median of 5,132,645 reads per sample. For sequence quality assurance and quality control (QA/QC), individual nucleotides were scored for each sequence, and bases were assigned individual Phred-scaled quality scores above 20 (Q> 20), which were considered in the subsequent variant detection pipeline, respectively (Fig. S. 1).

Oak genome assembly PM1N (https://urgi.versailles.inra.fr/download/oak/Qrob_PM1N.fa.gz) was used as the reference genome. The bioinformatics analysis as 'alignment to reference genome sequence' and 'variant calling' tools available in the software BOWTIE version bowtie−1.1.1 (Langmead et al. 2009), BWA version: bwa−0.6.1 (Li and Durbin, 2009), SAMTOOLS Version: samtools−0.1.16 (Li et al. 2009) and VELVET Version: velvet_1.2.10 (Zerbino and Birney 2008) were used. For variant calling, the three filtering protocols relaxed, standard and stringent were applied: (1) relaxed: minimum

sequencing depth per sample 6×, minimum Phred-scaled genotype quality per sample 10 and minimum percent of population genotyped 75 %; (2) standard: minimum sequencing depth per sample 10×, minimum Phred-scaled genotype quality per sample 13 and minimum percent of population genotyped 82.5 %; (3) stringent: minimum sequencing depth per sample 15×, minimum Phred-scaled genotype quality per sample 20 and minimum percent of population genotyped 90 %. Subsequently, the genotypes resulting from these analyses were cataloged in VCF format and could then be converted to JoinMap format. We calculated the finale maternal and paternal linkage maps using the standard data set.

### 5.2.3 Construction of linkage maps

Separate male and female linkage maps were constructed using markers in the two-way *pseudo-testcross* mapping configuration (Grattapaglia and Sederoff 1994) in the 249 crossing progenies. These so-called pseudo-testcross markers are markers that have certain alleles that are heterozygous in one parent and absent in the other. This means that the corresponding allele inherited from the heterozygous parent splits 1:1 in the progeny. Linkage maps were calculated by mapping markers that segregate in a 1:1 ratio (testcross configuration) using JoinMap 4.0 (Van Ooijen 2006). Markers that deviated statistically significantly from the expected 1:1 ratio were referred to as distorted markers. The obtained markers of the standard dataset were entered into JoinMap under the type "CP" (outbreeder full-sib family). Markers were assigned to linkage groups consisting of groups of ≥ 10 markers and recombination frequencies between 0.05 and 0.25 using the JoinMap 4 - function *'create groups using the grouping tree'* (Van Ooijen 2006). Regression mapping with the standard settings of a maximum recombination frequency of 0.40, a minimum LOD score of 1.0 and a goodness-of-fit jump threshold of 5.0 for removal of loci was used to determine the order of markers within each linkage group. *Kosambi's* mapping function (Kosambi 1943) was applied to transform recombination frequencies into centiMorgan (cM). Segregation distortion was assessed for individual markers using chi-squared goodness-of-fit tests ($X^2$) and distorted markers ($p<0.05$), deviating with statistical significance from the expected 1:1 ratio, were plotted on male and female linkage maps and highlighted with a star (Fig. 1). Linkage groups were assigned to individual chromosomes of the whole genome sequences using the physical position of the mapped markers.

Applying the software MapChart version 2.32 (Voorrips 2002), the male and female linkage maps were plotted.

## 5.3 Results and Discussion

The genetic map of the cross *Q. robur* subsp. *robur* x *Q. robur* subsp. *slavonica* generated in this study was constructed using the pseudo-testcross mapping strategy (Grattapaglia and Sederoff 1994) based on the use of the F1 generation. This mapping strategy is well suited for tree species because of their long generation times and outcrossing reproductive strategies. Since oaks, such as other forest trees, generally have a high degree of heterozygosity, the F1 generation contains a sufficient number of mapping pseudo-testcross markers. However, the cross used for this mapping is an intra-species cross with two genetically closely related parent trees.

Out of 1379 SNPs, 650 were in pseudo-testcross mapping configuration for the female and 729 for the male parent. The resulting female map was composed of 473 SNP markers and assigned to the 12 linkage groups (LG1f to LG12f). The number of SNP markers per LG ranged from 17 (LG9f) to 56 (LG8f), covering with individual linkage range from 22.7 (LG7f) to 121.8 cM (LG2f). The total map length was 885.0 cM, with one SNP marker per 1.87 cM on average. Whereas the total number of SNP markers mapped on the male map was 502 segregated in the testcross configuration (LG1m to LG12m) resulting in a map length of 898.4 cM and an average marker spacing of 1.79 cM. The number of markers per male LG ranged from 15 (LG7m) to 117 (LG8m), covering with individual linkage range from 15.5 (LG7m) to 119.8 cM (LG2m). Another comparative study from Bodénès et al. (2016) based on SNP markers in an intraspecific cross of *Q. robur* in France, the female linkage map consists of 1,170 SNP markers and the male of 1,137 SNPs with a total map length of 689 cM and 781 cM as well as an average marker spacing of 1.46 cM and 1.70 cM, respectively. Regarding the number of markers per linkage group, male LG ranges from 40 (LG4m) to 179 (LG2m) and female LG from 61 (LG4f) to 200 (LG2f), covering with individual linkage range from 54 cM (LG1m) to 102 cM (LG2m) and 50 cM (LG11f, LG12f) to 89 (LG2f), respectively (Bodénès et al. 2016). While Gailing et al. (2013) constructed a linkage map on the same intraspecific cross (*Q. robur* subsp. *robur* x *Q. robur* subsp. *slavonica*) however, they used microsatellites (SSRs and EST-SSRs) which result in a low-density map and thus have both a lower marker number (from 10 (LG12f) to 25 (LG2f) on the female and from 7 (LG7m) to 24 (LG2m) on the male map) and a greater spacing between markers (female map: 5.7 cM, male: 5.8 cM).

The number of linkage groups (LGs) corresponded to the number of haploid chromosomes of the species *Quercus* (n=12) in both maps. The correspondence of LGs to chromosome number in oaks may suggest that this may be a saturated map. However, the LGs would also need to be tested for the absence of uncoupled markers. The length of LGs in centiMorgan (cM) and the number of markers per LG were highly and significantly correlated for the female map (r=0.63, p=0.00002) as well as for the male map (r=0.73, p=0.0001). Number of markers, the length of linkage groups, and the average marker spacing were highly – but not significant – correlated between male and female map with the

lowest correlation found for the average marker spacing (r=0.67, p=0.355; r=0.87, p=0.399; r=0.61, p=0.278). It's interesting that the correlation was significant within the groups male and female, but not between groups. The lack of significance can indicate that the correlation is high by random chance, due to small sample size, outliers or non-linear relationship between the data. Such high but significant correlations between male and female map could be found in Gailing et al. (2013) (Number of markers: r=0.80, p=0.0009, the length of linkage groups: r=0.57, p=0.03, and the average marker spacing: r=0.80, p=0.0009).

Markers with a significant deviation from the expected Mendelian segregation ratios (segregation distortion, distorted markers) occurred on both the maternal and paternal linkage maps (Fig.1). Segregation distortion ($p < 0.05$) was found for single markers and groups of markers (24.8 % of all markers) on all linkage groups. A very similar segregation distortion of all markers (22.7%) was found in Gailing et al. (2013). Chromosomal regions showing segregation distortion on male (173 distorted markers at $p < 0.05$) and female (69 distorted markers at $p < 0.05$) linkage groups differed (Fig. 1). Larger groups of linked markers (n>3) with segregation distortion were found on LG1, LG2, LG3m, LG4, LG5m, LG6f, LG8m, LG10m, LG11m and LG12. In this study we also observed three types of segregation distortions, as in Bodénès et al. (2016): (1) Segregation distortions at the ends of LG: LG2m, LG7f, LG8f, LG9m, LG10m; (2) Segregation distortions in the middle of the LG: LG5, LG6, LG7m, LG9f, LG12 and (3) segregation distortions encompassing whole LG: LG1, LG2, LG3, LG4, LG8m, LG10f, LG11. The most significant distortions on the male map were observed for LG11f with 86.36 % of loci displaying segregation distortion, followed by LG5 (48.72 %), LG8 (47.01 %) and LG1 (45.95 %), whereas on the female map only LG6 shows the most significant segregation distortion with 60 % of markers. Of the remaining LGs, 3 LGs (LG3m, LG12m, LG1f) display segregation distortions for less than 45 % of markers, 6 LGs (LG4m, LG9m, LG1f, LG2f, LG4f, and LG10f) display segregation distortions for less than 25 % of markers, and the remaining 11 LGs display segregation distortions for less than 10 % of markers (Table S.1). Such distorted markers can result from either evaluation errors, PCR errors, randomly due to a relatively small mapping population, and linkage of certain markers to genes under selection, such as resistance genes (Bradshaw and Stettler 1994). However, adjacent markers should then also exhibit some degree of distortion. Clustering of distorted markers may highlight genomic regions containing genes under the influence of selection. Therefore, further analysis would need to identify the distorted genomic regions and their underlying genes.

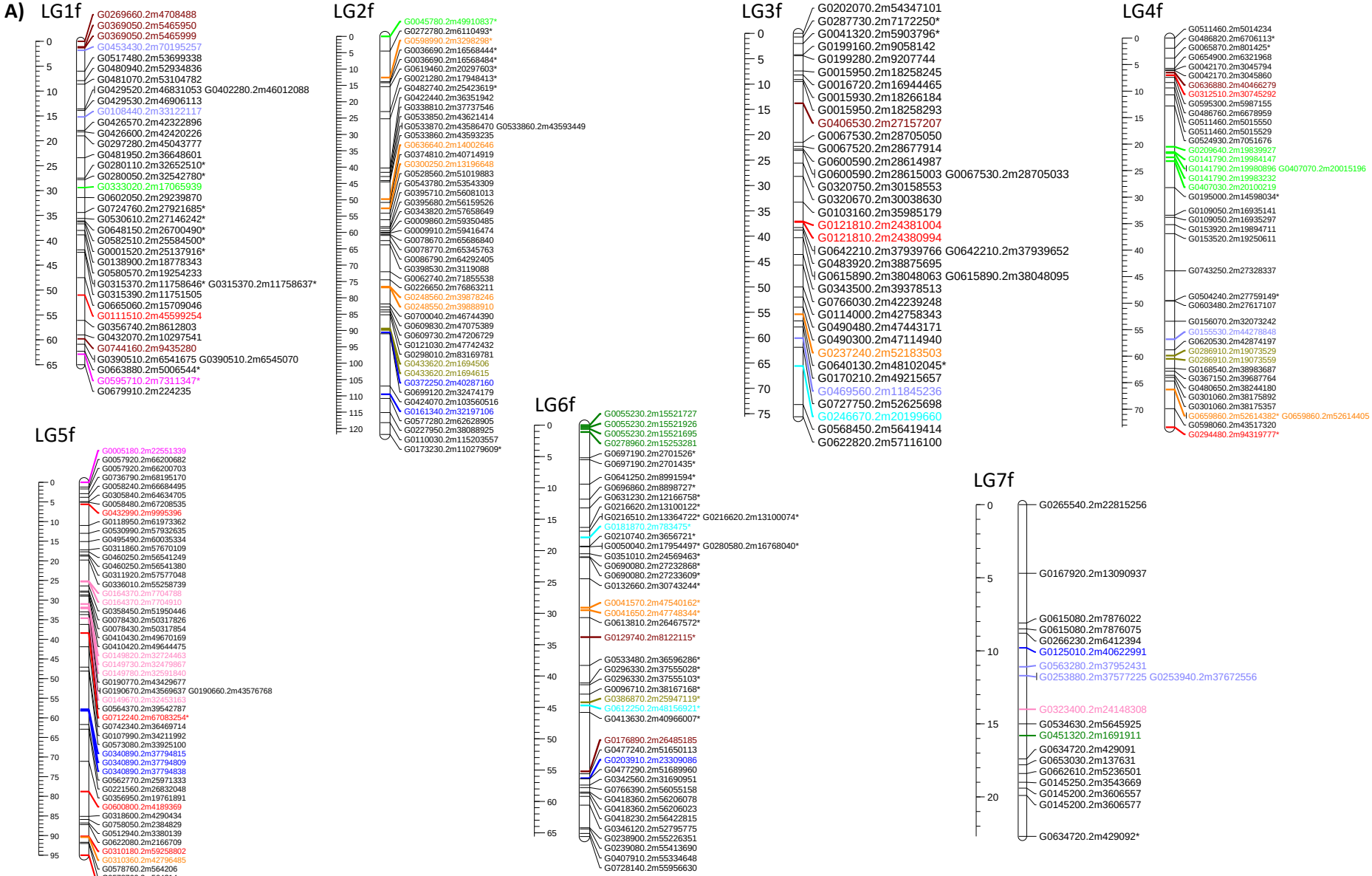
SNP markers, like SSR markers, are also suitable for aligning different genetic maps to each other, but these linkage maps are the first based on RADseq markers, thus no comparative genetic linkage maps are available in the literature for the oak genus based on the genome sequence. Therefore, unfortunately, due to the lack of identically mapped markers, collinearity comparisons

cannot be made with previous mapping studies (Barreneche et al. 1998, Bodénès et al. 2012, Bodénès et al. 2016, Gailing et al. 2013) to compare map positions in oak. Future analyses would need to attempt to determine the genomic position of the markers (SNPs, SSRs) for subsequent comparison. Furthermore, besides SNP markers, SSRs should also be used in the future, which are present in both male and female linkage maps and have already been used to align linkage maps in previous studies (Gailing et al. 2013, Bodénès et al. 2012).

Figure 1 also shows that some markers (highlighted with color), which are actually located on a different chromosome, were assigned to the wrong linkage group. Thus, the genomic position of the markers was determined. For example, the markers Qrob_441, Qrob_442 and Qrob_443 are actually located on chromosome 8 but were assigned to LG1 on the female map. A possible reason for marker groups of other chromosomes at the beginning and end of the linkage group (dispersal), like Qrob_174 to Qrob_177 on LG6, Qrob_416 to Qrob_418 on LG10f and Qrob_441 to Qrob_443 on LG1, could be the recombination frequency (statistical settings) (Fig.1). Furthermore, a low number of markers may result in certain markers being physically located on a chromosome but not on a linkage group in the genetic map. Due to low marker number, some markers could be physically located so far away from the next marker that, due to high recombination probabilities, linkage of the markers can no longer be detected.

## 5.4 Conclusion and outlook

In conclusion, the present study displayed a high density genetic linkage map of the cross *Q. robur* subsp. *robur* x *Q. robur* subsp. *slavonica* based on single nucleotide polymorphisms (SNPs) marker using the RADseq approach for construction. Genetic linkage maps should be used in further analysis as essential tools to localize chromosomal regions, quantitative trait loci (QTL), that are associated with quantitative trait variation. Specifically, the availability of a large number of SNP markers distributed in specific regions across the oak genome would serve as a basis for identifying QTL (QTL mapping) and candidate genes for yield-increasing traits, such as growth, budburst, leaf fall, stomatal density, water use efficiency, and leaf morphological traits. In addition, significant SNP marker associated with genes and QTLs for yield-increasing and adaptive traits could be used to select appropriate SNPs for genotyping. This SNP genotyping will be used to determine adaptive genetic variation within Slavonian and indigenous oak stands and to differentiate between these stands, as well as for marker-assisted selection. Since marker-assisted selection can be used at an early stage of plant development (seedling), the breeding process becomes faster and more efficient. This enables rapid decision support as well as recommendations in the choice of suitable provenances for forestry practice, which is particularly needed due to ongoing climate change.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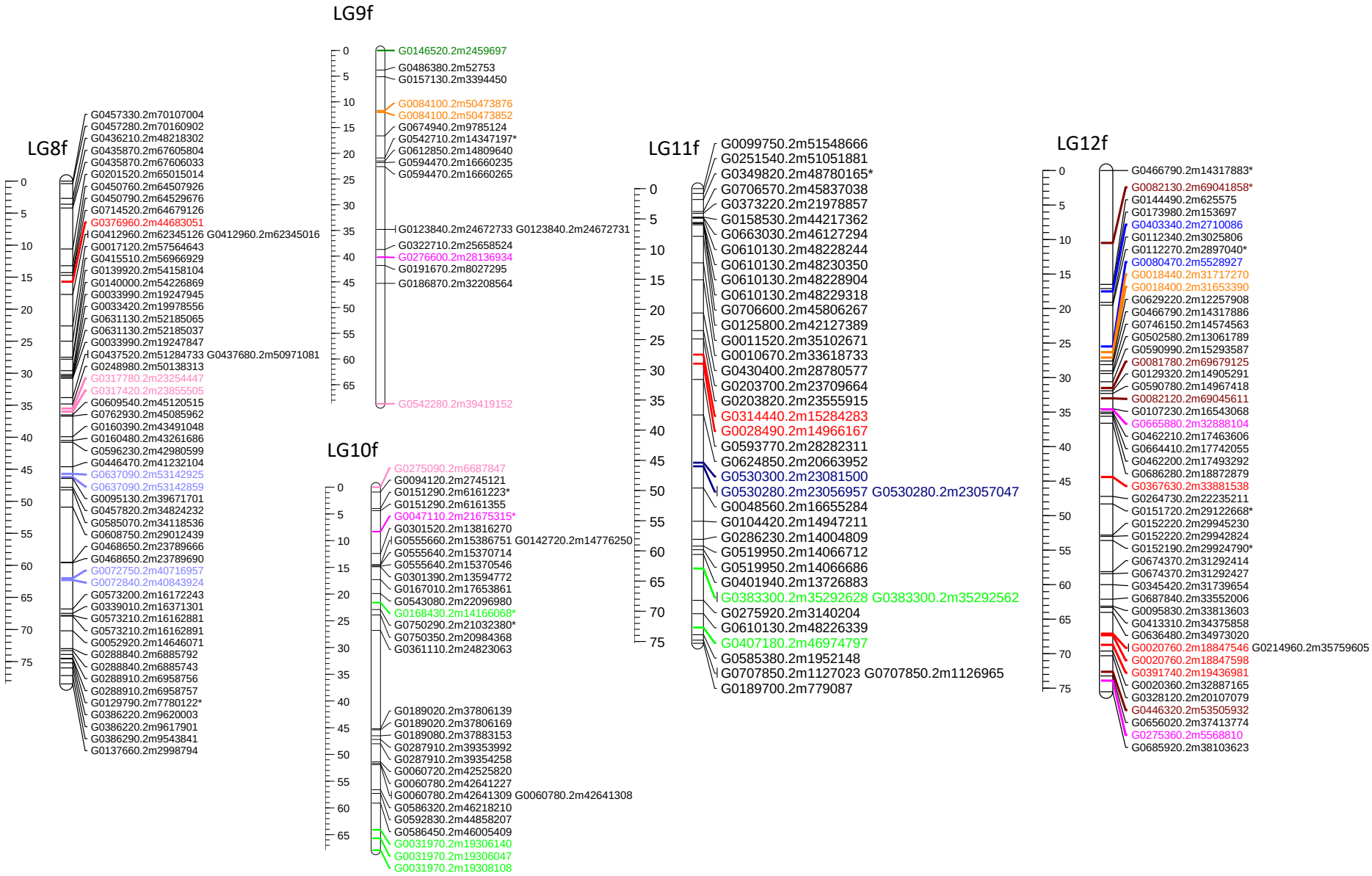
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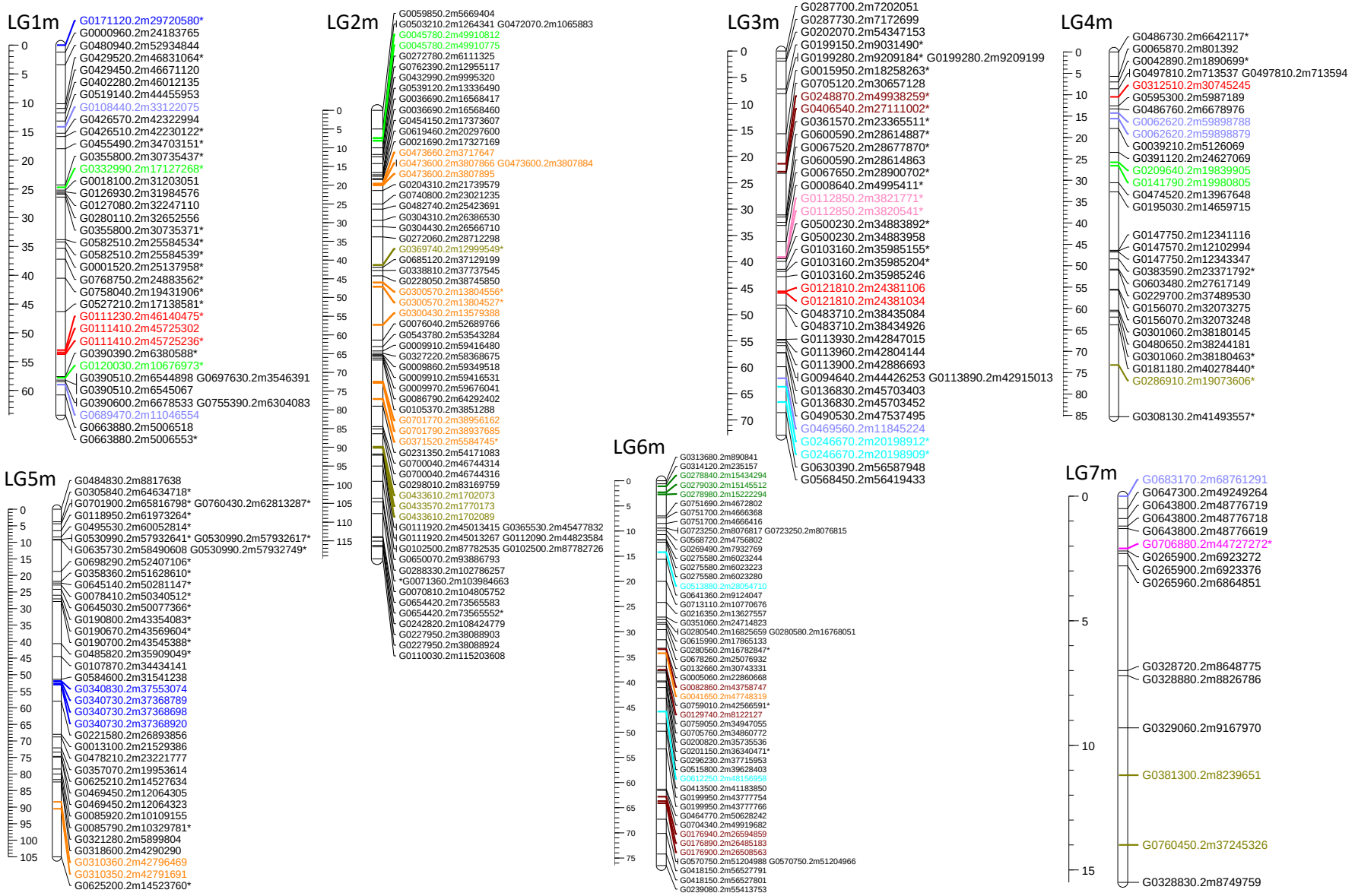
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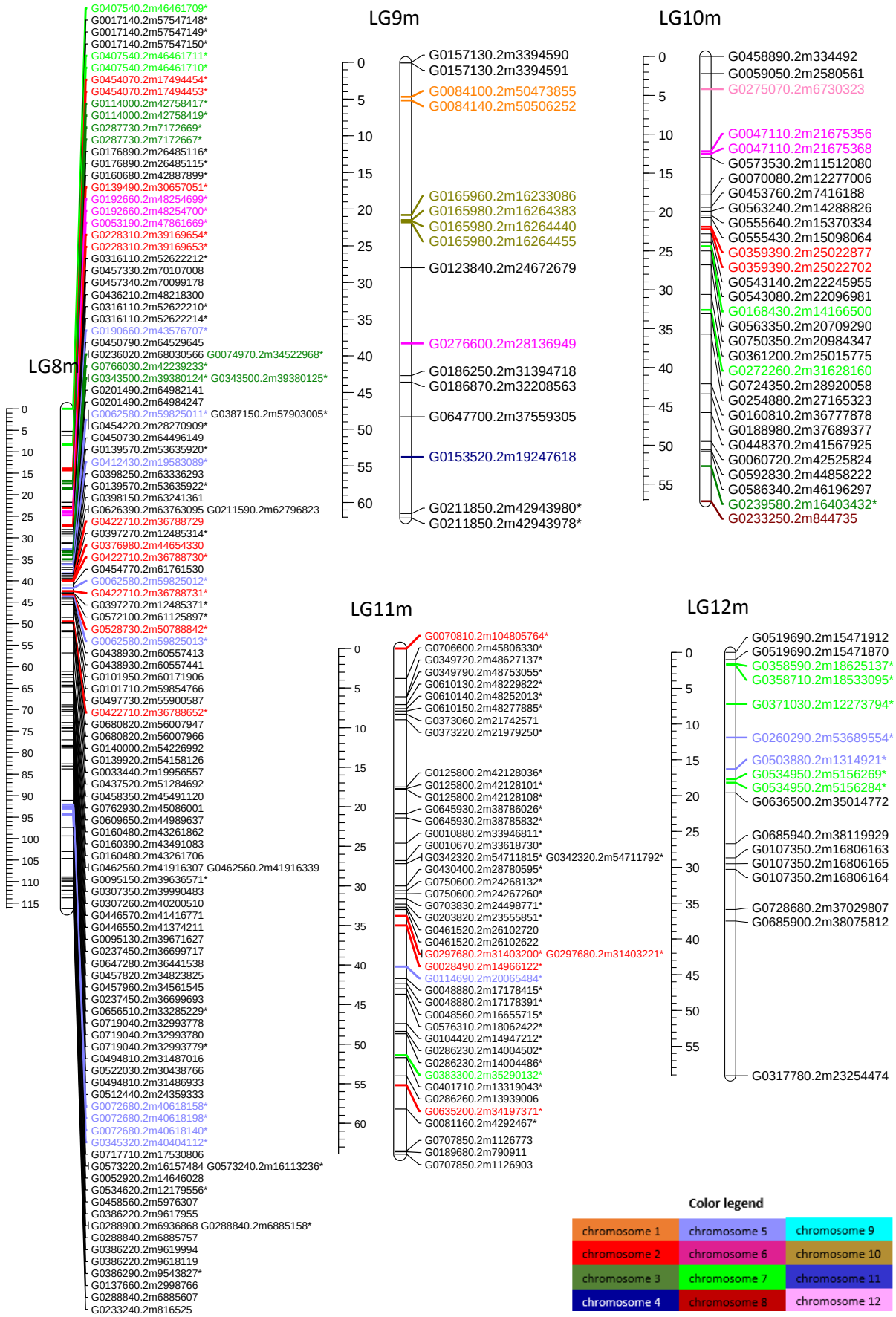


**Figure 1** (A) Female (f) and (B) male (m) linkage map of *Q. robur* fullsib family. Map distance between RADSeq markers is shown in centiMorgan (cM). Markers with significant segregation distortion are labeled (*p<0.05). Markers highlighted in color occur on chromosomes that differ from the linkage group shown based on the genome assembly.

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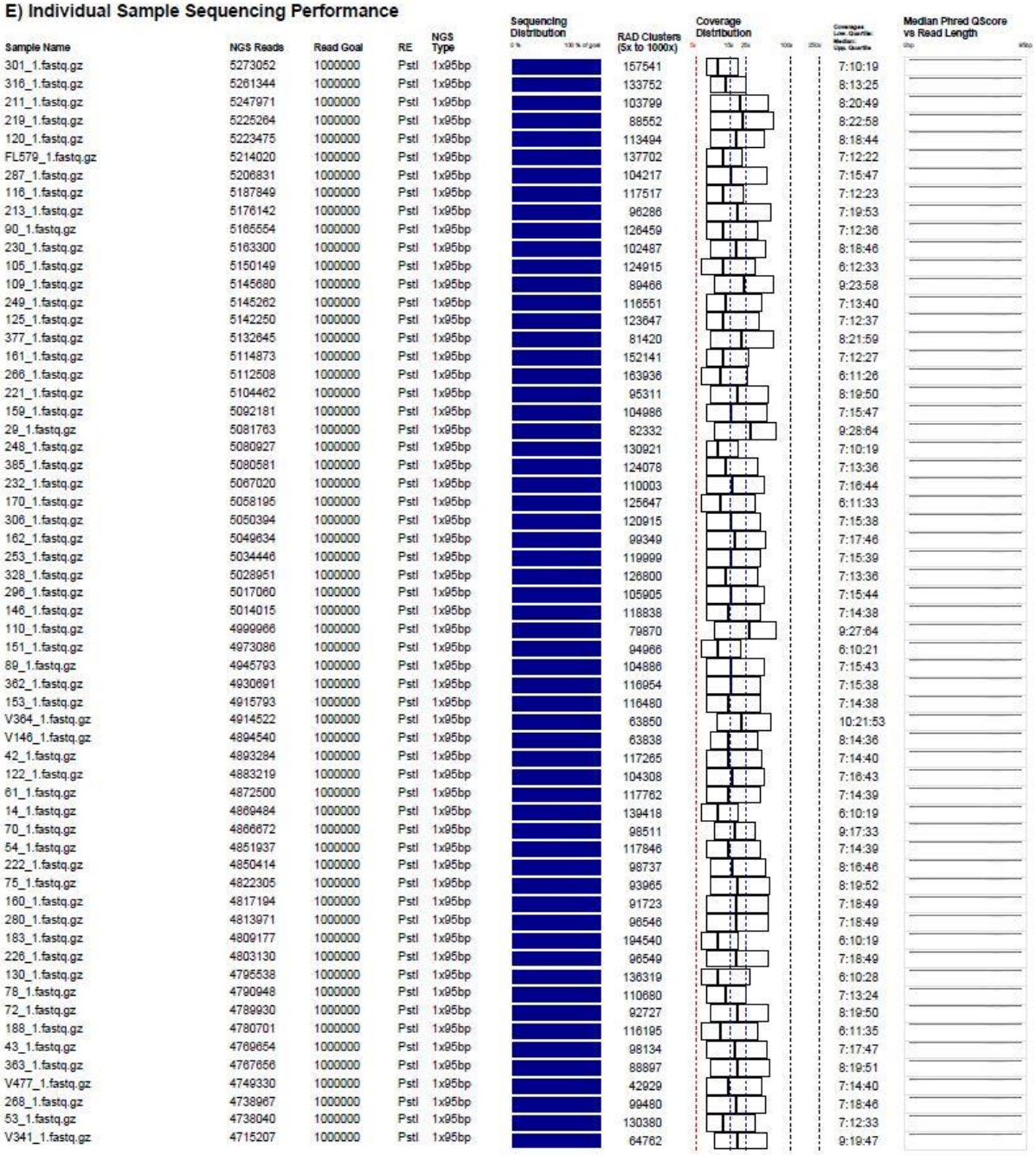
Kapitel 5

# Supplementary Material

## E) Individual Sample Sequencing Performance

| Sample Name | NGS Reads | Read Goal | RE | NGS Type | Sequencing Distribution | RAD Clusters (5x to 1000x) | Coverage Distribution | Coverage Low Quartile: Median: Upp. Quartile | Median Phred QScore vs Read Length |
|---|---|---|---|---|---|---|---|---|---|
| MB_1.fastq.gz | 174111181 | 1000000 | PstI | 1x95bp | | 833323 | | 6:9:19 | |
| VB_1.fastq.gz | 136427290 | 1000000 | PstI | 1x95bp | | 1122366 | | 7:11:25 | |
| FGXCONTROL_1.fastq.gz | 42360580 | 330000 | PstI | 1x95bp | | 151718 | | 6:10:19 | |
| 195_1.fastq.gz | 7396341 | 1000000 | PstI | 1x95bp | | 133722 | | 6:11:26 | |
| 322_1.fastq.gz | 7000364 | 1000000 | PstI | 1x95bp | | 163902 | | 6:9:19 | |
| 207_1.fastq.gz | 6978590 | 1000000 | PstI | 1x95bp | | 142434 | | 6:10:22 | |
| 112_1.fastq.gz | 6870844 | 1000000 | PstI | 1x95bp | | 213272 | | 6:9:16 | |
| 41_1.fastq.gz | 6795855 | 1000000 | PstI | 1x95bp | | 88159 | | 9:25:75 | |
| 197_1.fastq.gz | 6701237 | 1000000 | PstI | 1x95bp | | 168993 | | 6:9:17 | |
| 27_1.fastq.gz | 6672482 | 1000000 | PstI | 1x95bp | | 152146 | | 7:12:38 | |
| 380_1.fastq.gz | 6633179 | 1000000 | PstI | 1x95bp | | 142504 | | 6:10:22 | |
| 60_1.fastq.gz | 6620536 | 1000000 | PstI | 1x95bp | | 138825 | | 7:10:21 | |
| FL225_1.fastq.gz | 6582345 | 1000000 | PstI | 1x95bp | | 118346 | | 11:26:57 | |
| 95_1.fastq.gz | 6461392 | 1000000 | PstI | 1x95bp | | 175044 | | 7:10:18 | |
| 141_1.fastq.gz | 6377768 | 1000000 | PstI | 1x95bp | | 105423 | | 7:18:57 | |
| 294_1.fastq.gz | 6354557 | 1000000 | PstI | 1x95bp | | 111020 | | 6:10:24 | |
| 371_1.fastq.gz | 6312061 | 1000000 | PstI | 1x95bp | | 172361 | | 7:10:20 | |
| 3_1.fastq.gz | 6293460 | 1000000 | PstI | 1x95bp | | 131738 | | 6:8:16 | |
| 196_1.fastq.gz | 6270907 | 1000000 | PstI | 1x95bp | | 142195 | | 6:9:19 | |
| 104_1.fastq.gz | 6267393 | 1000000 | PstI | 1x95bp | | 96004 | | 9:25:65 | |
| 1_1.fastq.gz | 6257855 | 1000000 | PstI | 1x95bp | | 90792 | | 8:23:68 | |
| 142_1.fastq.gz | 6252884 | 1000000 | PstI | 1x95bp | | 101061 | | 8:19:61 | |
| 49_1.fastq.gz | 6227707 | 1000000 | PstI | 1x95bp | | 115855 | | 6:14:50 | |
| 7_1.fastq.gz | 6209686 | 1000000 | PstI | 1x95bp | | 129529 | | 7:13:42 | |
| 96_1.fastq.gz | 6188204 | 1000000 | PstI | 1x95bp | | 160448 | | 7:11:21 | |
| 92_1.fastq.gz | 6163379 | 1000000 | PstI | 1x95bp | | 89896 | | 8:22:66 | |
| 98_1.fastq.gz | 6151173 | 1000000 | PstI | 1x95bp | | 154167 | | 7:12:22 | |
| 325_1.fastq.gz | 6137875 | 1000000 | PstI | 1x95bp | | 88167 | | 9:25:69 | |
| 285_1.fastq.gz | 6091385 | 1000000 | PstI | 1x95bp | | 115390 | | 7:16:49 | |
| 28_1.fastq.gz | 6084070 | 1000000 | PstI | 1x95bp | | 139322 | | 6:10:24 | |
| 244_1.fastq.gz | 6080789 | 1000000 | PstI | 1x95bp | | 138520 | | 7:11:21 | |
| 239_1.fastq.gz | 6071779 | 1000000 | PstI | 1x95bp | | 87597 | | 8:21:65 | |
| 117_1.fastq.gz | 6051882 | 1000000 | PstI | 1x95bp | | 262148 | | 6:9:17 | |
| 231_1.fastq.gz | 6037755 | 1000000 | PstI | 1x95bp | | 137038 | | 7:15:39 | |
| 184_1.fastq.gz | 6036084 | 1000000 | PstI | 1x95bp | | 124444 | | 6:12:42 | |
| 143_1.fastq.gz | 6015485 | 1000000 | PstI | 1x95bp | | 170339 | | 6:10:19 | |
| 36_1.fastq.gz | 6006899 | 1000000 | PstI | 1x95bp | | 139809 | | 6:10:19 | |
| 168_1.fastq.gz | 5998099 | 1000000 | PstI | 1x95bp | | 110985 | | 7:11:25 | |
| 79_1.fastq.gz | 5988539 | 1000000 | PstI | 1x95bp | | 132053 | | 8:15:41 | |
| 37_1.fastq.gz | 5966455 | 1000000 | PstI | 1x95bp | | 142517 | | 7:13:35 | |
| 81_1.fastq.gz | 5957016 | 1000000 | PstI | 1x95bp | | 133720 | | 6:10:19 | |
| 373_1.fastq.gz | 5956104 | 1000000 | PstI | 1x95bp | | 82830 | | 8:20:70 | |
| 217_1.fastq.gz | 5954308 | 1000000 | PstI | 1x95bp | | 98682 | | 9:20:57 | |
| 323_1.fastq.gz | 5951636 | 1000000 | PstI | 1x95bp | | 98204 | | 8:19:54 | |
| 365_1.fastq.gz | 5942720 | 1000000 | PstI | 1x95bp | | 155273 | | 7:10:20 | |
| 63_1.fastq.gz | 5935094 | 1000000 | PstI | 1x95bp | | 119202 | | 7:10:20 | |
| 19_1.fastq.gz | 5922288 | 1000000 | PstI | 1x95bp | | 162636 | | 6:8:16 | |
| 240_1.fastq.gz | 5873632 | 1000000 | PstI | 1x95bp | | 131837 | | 7:13:39 | |
| 138_1.fastq.gz | 5871839 | 1000000 | PstI | 1x95bp | | 177726 | | 6:11:26 | |
| 264_1.fastq.gz | 5843716 | 1000000 | PstI | 1x95bp | | 119068 | | 7:15:43 | |
| 32_1.fastq.gz | 5842695 | 1000000 | PstI | 1x95bp | | 85135 | | 8:22:66 | |
| 374_1.fastq.gz | 5841288 | 1000000 | PstI | 1x95bp | | 132442 | | 6:9:17 | |
| 199_1.fastq.gz | 5829844 | 1000000 | PstI | 1x95bp | | 122789 | | 6:10:22 | |
| 272_1.fastq.gz | 5826312 | 1000000 | PstI | 1x95bp | | 148084 | | 6:10:18 | |
| 156_1.fastq.gz | 5824382 | 1000000 | PstI | 1x95bp | | 114109 | | 7:14:47 | |
| 83_1.fastq.gz | 5814943 | 1000000 | PstI | 1x95bp | | 120041 | | 7:15:43 | |
| 76_1.fastq.gz | 5812618 | 1000000 | PstI | 1x95bp | | 140346 | | 7:12:22 | |
| 237_1.fastq.gz | 5792364 | 1000000 | PstI | 1x95bp | | 106017 | | 8:21:54 | |
| 332_1.fastq.gz | 5789857 | 1000000 | PstI | 1x95bp | | 129965 | | 7:13:40 | |
| 86_1.fastq.gz | 5773596 | 1000000 | PstI | 1x95bp | | 110122 | | 9:19:47 | |

## E) Individual Sample Sequencing Performance



| Sample Name | NGS Reads | Read Goal | RE | NGS Type | Sequencing Distribution | RAD Clusters (5x to 1000x) | Coverage Distribution | Coverage Low Quartile: Median: Upp. Quartile | Median Phred QScore vs Read Length |
|---|---|---|---|---|---|---|---|---|---|
| 301_1.fastq.gz | 5273052 | 1000000 | PstI | 1x95bp | | 157541 | | 7:10:19 | |
| 316_1.fastq.gz | 5261344 | 1000000 | PstI | 1x95bp | | 133752 | | 8:13:25 | |
| 211_1.fastq.gz | 5247971 | 1000000 | PstI | 1x95bp | | 103799 | | 8:20:49 | |
| 219_1.fastq.gz | 5225264 | 1000000 | PstI | 1x95bp | | 88552 | | 8:22:58 | |
| 120_1.fastq.gz | 5223475 | 1000000 | PstI | 1x95bp | | 113494 | | 8:18:44 | |
| FL579_1.fastq.gz | 5214020 | 1000000 | PstI | 1x95bp | | 137702 | | 7:12:22 | |
| 287_1.fastq.gz | 5206831 | 1000000 | PstI | 1x95bp | | 104217 | | 7:15:47 | |
| 116_1.fastq.gz | 5187849 | 1000000 | PstI | 1x95bp | | 117517 | | 7:12:23 | |
| 213_1.fastq.gz | 5176142 | 1000000 | PstI | 1x95bp | | 96286 | | 7:19:53 | |
| 90_1.fastq.gz | 5165554 | 1000000 | PstI | 1x95bp | | 126459 | | 7:12:36 | |
| 230_1.fastq.gz | 5163300 | 1000000 | PstI | 1x95bp | | 102487 | | 8:18:46 | |
| 105_1.fastq.gz | 5150149 | 1000000 | PstI | 1x95bp | | 124915 | | 6:12:33 | |
| 109_1.fastq.gz | 5145680 | 1000000 | PstI | 1x95bp | | 89466 | | 9:23:58 | |
| 249_1.fastq.gz | 5145262 | 1000000 | PstI | 1x95bp | | 116551 | | 7:13:40 | |
| 125_1.fastq.gz | 5142250 | 1000000 | PstI | 1x95bp | | 123647 | | 7:12:37 | |
| 377_1.fastq.gz | 5132645 | 1000000 | PstI | 1x95bp | | 81420 | | 8:21:59 | |
| 161_1.fastq.gz | 5114873 | 1000000 | PstI | 1x95bp | | 152141 | | 7:12:27 | |
| 266_1.fastq.gz | 5112508 | 1000000 | PstI | 1x95bp | | 163936 | | 6:11:26 | |
| 221_1.fastq.gz | 5104462 | 1000000 | PstI | 1x95bp | | 95311 | | 8:19:50 | |
| 159_1.fastq.gz | 5092181 | 1000000 | PstI | 1x95bp | | 104986 | | 7:15:47 | |
| 29_1.fastq.gz | 5081763 | 1000000 | PstI | 1x95bp | | 82332 | | 9:28:64 | |
| 248_1.fastq.gz | 5080927 | 1000000 | PstI | 1x95bp | | 130921 | | 7:10:19 | |
| 385_1.fastq.gz | 5080581 | 1000000 | PstI | 1x95bp | | 124078 | | 7:13:36 | |
| 232_1.fastq.gz | 5067020 | 1000000 | PstI | 1x95bp | | 110003 | | 7:16:44 | |
| 170_1.fastq.gz | 5058195 | 1000000 | PstI | 1x95bp | | 125647 | | 6:11:33 | |
| 306_1.fastq.gz | 5050394 | 1000000 | PstI | 1x95bp | | 120915 | | 7:15:38 | |
| 162_1.fastq.gz | 5049634 | 1000000 | PstI | 1x95bp | | 99349 | | 7:17:46 | |
| 253_1.fastq.gz | 5034446 | 1000000 | PstI | 1x95bp | | 119999 | | 7:15:39 | |
| 328_1.fastq.gz | 5028951 | 1000000 | PstI | 1x95bp | | 126800 | | 7:13:36 | |
| 296_1.fastq.gz | 5017060 | 1000000 | PstI | 1x95bp | | 105905 | | 7:15:44 | |
| 146_1.fastq.gz | 5014015 | 1000000 | PstI | 1x95bp | | 118838 | | 7:14:38 | |
| 110_1.fastq.gz | 4999966 | 1000000 | PstI | 1x95bp | | 79870 | | 9:27:64 | |
| 151_1.fastq.gz | 4973086 | 1000000 | PstI | 1x95bp | | 94966 | | 6:10:21 | |
| 89_1.fastq.gz | 4945793 | 1000000 | PstI | 1x95bp | | 104886 | | 7:15:43 | |
| 362_1.fastq.gz | 4930691 | 1000000 | PstI | 1x95bp | | 116954 | | 7:15:38 | |
| 153_1.fastq.gz | 4915793 | 1000000 | PstI | 1x95bp | | 116480 | | 7:14:38 | |
| V364_1.fastq.gz | 4914522 | 1000000 | PstI | 1x95bp | | 63850 | | 10:21:53 | |
| V146_1.fastq.gz | 4894540 | 1000000 | PstI | 1x95bp | | 63838 | | 8:14:36 | |
| 42_1.fastq.gz | 4893284 | 1000000 | PstI | 1x95bp | | 117265 | | 7:14:40 | |
| 122_1.fastq.gz | 4883219 | 1000000 | PstI | 1x95bp | | 104308 | | 7:16:43 | |
| 61_1.fastq.gz | 4872500 | 1000000 | PstI | 1x95bp | | 117762 | | 7:14:39 | |
| 14_1.fastq.gz | 4869484 | 1000000 | PstI | 1x95bp | | 139418 | | 6:10:19 | |
| 70_1.fastq.gz | 4866672 | 1000000 | PstI | 1x95bp | | 98511 | | 9:17:33 | |
| 54_1.fastq.gz | 4851937 | 1000000 | PstI | 1x95bp | | 117846 | | 7:14:39 | |
| 222_1.fastq.gz | 4850414 | 1000000 | PstI | 1x95bp | | 98737 | | 8:16:46 | |
| 75_1.fastq.gz | 4822305 | 1000000 | PstI | 1x95bp | | 93965 | | 8:19:52 | |
| 160_1.fastq.gz | 4817194 | 1000000 | PstI | 1x95bp | | 91723 | | 7:18:49 | |
| 280_1.fastq.gz | 4813971 | 1000000 | PstI | 1x95bp | | 96546 | | 7:18:49 | |
| 183_1.fastq.gz | 4809177 | 1000000 | PstI | 1x95bp | | 194540 | | 6:10:19 | |
| 226_1.fastq.gz | 4803130 | 1000000 | PstI | 1x95bp | | 96549 | | 7:18:49 | |
| 130_1.fastq.gz | 4795538 | 1000000 | PstI | 1x95bp | | 136319 | | 6:10:28 | |
| 78_1.fastq.gz | 4790948 | 1000000 | PstI | 1x95bp | | 110680 | | 7:13:24 | |
| 72_1.fastq.gz | 4789930 | 1000000 | PstI | 1x95bp | | 92727 | | 8:19:50 | |
| 188_1.fastq.gz | 4780701 | 1000000 | PstI | 1x95bp | | 116195 | | 6:11:35 | |
| 43_1.fastq.gz | 4769654 | 1000000 | PstI | 1x95bp | | 98134 | | 7:17:47 | |
| 363_1.fastq.gz | 4767656 | 1000000 | PstI | 1x95bp | | 88897 | | 8:19:51 | |
| V477_1.fastq.gz | 4749330 | 1000000 | PstI | 1x95bp | | 42929 | | 7:14:40 | |
| 268_1.fastq.gz | 4738967 | 1000000 | PstI | 1x95bp | | 99480 | | 7:18:46 | |
| 53_1.fastq.gz | 4738040 | 1000000 | PstI | 1x95bp | | 130380 | | 7:12:33 | |
| V341_1.fastq.gz | 4715207 | 1000000 | PstI | 1x95bp | | 64762 | | 9:19:47 | |

## E) Individual Sample Sequencing Performance

| Sample Name | NGS Reads | Read Goal | RE | NGS Type | Sequencing Distribution | RAD Clusters (5x to 1000x) | Coverage Distribution | Median Phred QScore vs Read Length |
|---|---|---|---|---|---|---|---|---|
| 301_1.fastq.gz | 5273052 | 1000000 | PstI | 1x95bp | | 157541 | 7:10:19 | |
| 316_1.fastq.gz | 5261344 | 1000000 | PstI | 1x95bp | | 133752 | 8:13:25 | |
| 211_1.fastq.gz | 5247971 | 1000000 | PstI | 1x95bp | | 103799 | 8:20:49 | |
| 219_1.fastq.gz | 5225264 | 1000000 | PstI | 1x95bp | | 88552 | 8:22:58 | |
| 120_1.fastq.gz | 5223475 | 1000000 | PstI | 1x95bp | | 113494 | 8:18:44 | |
| FL579_1.fastq.gz | 5214020 | 1000000 | PstI | 1x95bp | | 137702 | 7:12:22 | |
| 287_1.fastq.gz | 5206831 | 1000000 | PstI | 1x95bp | | 104217 | 7:15:47 | |
| 116_1.fastq.gz | 5187849 | 1000000 | PstI | 1x95bp | | 117517 | 7:12:23 | |
| 213_1.fastq.gz | 5176142 | 1000000 | PstI | 1x95bp | | 96286 | 7:19:53 | |
| 90_1.fastq.gz | 5165554 | 1000000 | PstI | 1x95bp | | 126459 | 7:12:36 | |
| 230_1.fastq.gz | 5163300 | 1000000 | PstI | 1x95bp | | 102487 | 8:18:46 | |
| 105_1.fastq.gz | 5150149 | 1000000 | PstI | 1x95bp | | 124915 | 6:12:33 | |
| 109_1.fastq.gz | 5145680 | 1000000 | PstI | 1x95bp | | 89466 | 9:23:58 | |
| 249_1.fastq.gz | 5145262 | 1000000 | PstI | 1x95bp | | 116551 | 7:13:40 | |
| 125_1.fastq.gz | 5142250 | 1000000 | PstI | 1x95bp | | 123647 | 7:12:37 | |
| 377_1.fastq.gz | 5132645 | 1000000 | PstI | 1x95bp | | 81420 | 8:21:59 | |
| 161_1.fastq.gz | 5114873 | 1000000 | PstI | 1x95bp | | 152141 | 7:12:27 | |
| 266_1.fastq.gz | 5112508 | 1000000 | PstI | 1x95bp | | 163936 | 6:11:26 | |
| 221_1.fastq.gz | 5104462 | 1000000 | PstI | 1x95bp | | 95311 | 8:19:50 | |
| 159_1.fastq.gz | 5092181 | 1000000 | PstI | 1x95bp | | 104986 | 7:15:47 | |
| 29_1.fastq.gz | 5081763 | 1000000 | PstI | 1x95bp | | 82332 | 9:28:64 | |
| 248_1.fastq.gz | 5080927 | 1000000 | PstI | 1x95bp | | 130921 | 7:10:19 | |
| 385_1.fastq.gz | 5080581 | 1000000 | PstI | 1x95bp | | 124078 | 7:13:36 | |
| 232_1.fastq.gz | 5067020 | 1000000 | PstI | 1x95bp | | 110003 | 7:16:44 | |
| 170_1.fastq.gz | 5058195 | 1000000 | PstI | 1x95bp | | 125647 | 6:11:33 | |
| 306_1.fastq.gz | 5050394 | 1000000 | PstI | 1x95bp | | 120915 | 7:15:38 | |
| 162_1.fastq.gz | 5049634 | 1000000 | PstI | 1x95bp | | 99349 | 7:17:46 | |
| 253_1.fastq.gz | 5034446 | 1000000 | PstI | 1x95bp | | 119999 | 7:15:39 | |
| 328_1.fastq.gz | 5028951 | 1000000 | PstI | 1x95bp | | 126800 | 7:13:36 | |
| 296_1.fastq.gz | 5017060 | 1000000 | PstI | 1x95bp | | 105905 | 7:15:44 | |
| 146_1.fastq.gz | 5014015 | 1000000 | PstI | 1x95bp | | 118838 | 7:14:38 | |
| 110_1.fastq.gz | 4999966 | 1000000 | PstI | 1x95bp | | 79870 | 9:27:64 | |
| 151_1.fastq.gz | 4973086 | 1000000 | PstI | 1x95bp | | 94966 | 6:10:21 | |
| 89_1.fastq.gz | 4945793 | 1000000 | PstI | 1x95bp | | 104886 | 7:15:43 | |
| 362_1.fastq.gz | 4930691 | 1000000 | PstI | 1x95bp | | 116954 | 7:15:38 | |
| 153_1.fastq.gz | 4915793 | 1000000 | PstI | 1x95bp | | 116480 | 7:14:38 | |
| V364_1.fastq.gz | 4914522 | 1000000 | PstI | 1x95bp | | 63850 | 10:21:53 | |
| V146_1.fastq.gz | 4894540 | 1000000 | PstI | 1x95bp | | 63838 | 8:14:36 | |
| 42_1.fastq.gz | 4893284 | 1000000 | PstI | 1x95bp | | 117265 | 7:14:40 | |
| 122_1.fastq.gz | 4883219 | 1000000 | PstI | 1x95bp | | 104308 | 7:16:43 | |
| 61_1.fastq.gz | 4872500 | 1000000 | PstI | 1x95bp | | 117762 | 7:14:39 | |
| 14_1.fastq.gz | 4869484 | 1000000 | PstI | 1x95bp | | 139418 | 6:10:19 | |
| 70_1.fastq.gz | 4866672 | 1000000 | PstI | 1x95bp | | 98511 | 9:17:33 | |
| 54_1.fastq.gz | 4851937 | 1000000 | PstI | 1x95bp | | 117846 | 7:14:39 | |
| 222_1.fastq.gz | 4850414 | 1000000 | PstI | 1x95bp | | 98737 | 8:16:46 | |
| 75_1.fastq.gz | 4822305 | 1000000 | PstI | 1x95bp | | 93965 | 8:19:52 | |
| 160_1.fastq.gz | 4817194 | 1000000 | PstI | 1x95bp | | 91723 | 7:18:49 | |
| 280_1.fastq.gz | 4813971 | 1000000 | PstI | 1x95bp | | 96546 | 7:18:49 | |
| 183_1.fastq.gz | 4809177 | 1000000 | PstI | 1x95bp | | 104540 | 6:10:19 | |
| 226_1.fastq.gz | 4803130 | 1000000 | PstI | 1x95bp | | 96549 | 7:18:49 | |
| 130_1.fastq.gz | 4795538 | 1000000 | PstI | 1x95bp | | 136319 | 6:10:28 | |
| 78_1.fastq.gz | 4790948 | 1000000 | PstI | 1x95bp | | 110680 | 7:13:24 | |
| 72_1.fastq.gz | 4789930 | 1000000 | PstI | 1x95bp | | 92727 | 8:19:50 | |
| 188_1.fastq.gz | 4780701 | 1000000 | PstI | 1x95bp | | 116195 | 6:11:35 | |
| 43_1.fastq.gz | 4769654 | 1000000 | PstI | 1x95bp | | 98134 | 7:17:47 | |
| 363_1.fastq.gz | 4767656 | 1000000 | PstI | 1x95bp | | 88897 | 8:19:51 | |
| V477_1.fastq.gz | 4749330 | 1000000 | PstI | 1x95bp | | 42929 | 7:14:40 | |
| 268_1.fastq.gz | 4738967 | 1000000 | PstI | 1x95bp | | 99480 | 7:18:46 | |
| 53_1.fastq.gz | 4738040 | 1000000 | PstI | 1x95bp | | 130380 | 7:12:33 | |
| V341_1.fastq.gz | 4715207 | 1000000 | PstI | 1x95bp | | 64762 | 9:19:47 | |

## E) Individual Sample Sequencing Performance

| Sample Name | NGS Reads | Read Goal | RE | NGS Type | Sequencing Distribution | RAD Clusters (5x to 1000x) | Coverage Distribution | Coverages Low. Quartile: Median: Upp. Quartile | Median Phred QScore vs Read Length |
|---|---|---|---|---|---|---|---|---|---|
| 68_1.fastq.gz | 4706967 | 1000000 | PstI | 1x95bp | | 118052 | | 7:12:36 | |
| 185_1.fastq.gz | 4696675 | 1000000 | PstI | 1x95bp | | 91928 | | 7:17:48 | |
| 26_1.fastq.gz | 4690322 | 1000000 | PstI | 1x95bp | | 134677 | | 6:12:31 | |
| 216_1.fastq.gz | 4685977 | 1000000 | PstI | 1x95bp | | 124530 | | 7:12:23 | |
| 210_1.fastq.gz | 4685566 | 1000000 | PstI | 1x95bp | | 143612 | | 6:10:23 | |
| 114_1.fastq.gz | 4657297 | 1000000 | PstI | 1x95bp | | 118689 | | 7:12:34 | |
| 259_1.fastq.gz | 4642470 | 1000000 | PstI | 1x95bp | | 109485 | | 8:15:29 | |
| 108_1.fastq.gz | 4638361 | 1000000 | PstI | 1x95bp | | 90488 | | 8:21:50 | |
| 57_1.fastq.gz | 4593737 | 1000000 | PstI | 1x95bp | | 113391 | | 7:13:38 | |
| 131_1.fastq.gz | 4590267 | 1000000 | PstI | 1x95bp | | 130274 | | 9:17:32 | |
| 214_1.fastq.gz | 4589704 | 1000000 | PstI | 1x95bp | | 145069 | | 6:10:26 | |
| 358_1.fastq.gz | 4587255 | 1000000 | PstI | 1x95bp | | 100173 | | 7:18:42 | |
| 252_1.fastq.gz | 4584358 | 1000000 | PstI | 1x95bp | | 117286 | | 7:13:35 | |
| 172_1.fastq.gz | 4566244 | 1000000 | PstI | 1x95bp | | 113774 | | 7:13:36 | |
| 129_1.fastq.gz | 4549458 | 1000000 | PstI | 1x95bp | | 88509 | | 8:21:50 | |
| 113_1.fastq.gz | 4547305 | 1000000 | PstI | 1x95bp | | 85549 | | 8:22:53 | |
| 12_1.fastq.gz | 4538588 | 1000000 | PstI | 1x95bp | | 119013 | | 7:14:36 | |
| 55_1.fastq.gz | 4538400 | 1000000 | PstI | 1x95bp | | 174030 | | 7:11:21 | |
| 304_1.fastq.gz | 4518459 | 1000000 | PstI | 1x95bp | | 80159 | | 9:27:57 | |
| V352_1.fastq.gz | 4504603 | 1000000 | PstI | 1x95bp | | 55297 | | 9:18:45 | |
| 208_1.fastq.gz | 4493594 | 1000000 | PstI | 1x95bp | | 151609 | | 7:13:25 | |
| 194_1.fastq.gz | 4485690 | 1000000 | PstI | 1x95bp | | 113342 | | 7:13:35 | |
| 145_1.fastq.gz | 4474508 | 1000000 | PstI | 1x95bp | | 116448 | | 6:13:34 | |
| 144_1.fastq.gz | 4473817 | 1000000 | PstI | 1x95bp | | 100086 | | 7:15:43 | |
| 13_1.fastq.gz | 4454018 | 1000000 | PstI | 1x95bp | | 108291 | | 6:10:22 | |
| 386_1.fastq.gz | 4444540 | 1000000 | PstI | 1x95bp | | 88119 | | 8:19:50 | |
| 236_1.fastq.gz | 4428349 | 1000000 | PstI | 1x95bp | | 109138 | | 7:12:37 | |
| 150_1.fastq.gz | 4409923 | 1000000 | PstI | 1x95bp | | 138366 | | 7:12:27 | |
| 173_1.fastq.gz | 4402497 | 1000000 | PstI | 1x95bp | | 111181 | | 7:14:36 | |
| 326_1.fastq.gz | 4394862 | 1000000 | PstI | 1x95bp | | 101105 | | 7:15:41 | |
| 133_1.fastq.gz | 4385115 | 1000000 | PstI | 1x95bp | | 102752 | | 7:16:40 | |
| 115_1.fastq.gz | 4380694 | 1000000 | PstI | 1x95bp | | 116111 | | 7:13:33 | |
| 366_1.fastq.gz | 4331066 | 1000000 | PstI | 1x95bp | | 135592 | | 7:12:28 | |
| 163_1.fastq.gz | 4324650 | 1000000 | PstI | 1x95bp | | 83980 | | 8:19:47 | |
| 312_1.fastq.gz | 4318680 | 1000000 | PstI | 1x95bp | | 102516 | | 7:15:40 | |
| 148_1.fastq.gz | 4314838 | 1000000 | PstI | 1x95bp | | 83300 | | 8:21:50 | |
| V340_1.fastq.gz | 4295158 | 1000000 | PstI | 1x95bp | | 51051 | | 10:23:57 | |
| V366_1.fastq.gz | 4291259 | 1000000 | PstI | 1x95bp | | 49394 | | 7:14:40 | |
| 134_1.fastq.gz | 4280935 | 1000000 | PstI | 1x95bp | | 147132 | | 7:12:24 | |
| 169_1.fastq.gz | 4279910 | 1000000 | PstI | 1x95bp | | 64502 | | 8:16:35 | |
| 324_1.fastq.gz | 4260992 | 1000000 | PstI | 1x95bp | | 93607 | | 8:15:31 | |
| 283_1.fastq.gz | 4253376 | 1000000 | PstI | 1x95bp | | 102910 | | 7:15:40 | |
| 311_1.fastq.gz | 4226831 | 1000000 | PstI | 1x95bp | | 109390 | | 7:13:36 | |
| 276_1.fastq.gz | 4224052 | 1000000 | PstI | 1x95bp | | 105363 | | 7:15:38 | |
| 190_1.fastq.gz | 4222144 | 1000000 | PstI | 1x95bp | | 93303 | | 8:20:45 | |
| 202_1.fastq.gz | 4203450 | 1000000 | PstI | 1x95bp | | 86461 | | 8:19:46 | |
| 223_1.fastq.gz | 4197919 | 1000000 | PstI | 1x95bp | | 99322 | | 7:17:41 | |
| V338_1.fastq.gz | 4187601 | 1000000 | PstI | 1x95bp | | 58365 | | 8:17:44 | |
| 340_1.fastq.gz | 4179062 | 1000000 | PstI | 1x95bp | | 105622 | | 7:14:36 | |
| 279_1.fastq.gz | 4159457 | 1000000 | PstI | 1x95bp | | 98048 | | 7:15:40 | |
| 119_1.fastq.gz | 4148568 | 1000000 | PstI | 1x95bp | | 77715 | | 8:16:34 | |
| 56_1.fastq.gz | 4144303 | 1000000 | PstI | 1x95bp | | 79883 | | 9:16:32 | |
| 174_1.fastq.gz | 4142181 | 1000000 | PstI | 1x95bp | | 102821 | | 7:15:37 | |
| 127_1.fastq.gz | 4135022 | 1000000 | PstI | 1x95bp | | 103511 | | 8:16:38 | |
| 97_1.fastq.gz | 4118444 | 1000000 | PstI | 1x95bp | | 113142 | | 7:12:31 | |
| 192_1.fastq.gz | 4110350 | 1000000 | PstI | 1x95bp | | 142891 | | 6:10:22 | |
| 209_1.fastq.gz | 4084015 | 1000000 | PstI | 1x95bp | | 98761 | | 7:14:38 | |
| 225_1.fastq.gz | 4082273 | 1000000 | PstI | 1x95bp | | 89067 | | 8:19:46 | |
| 348_1.fastq.gz | 4046324 | 1000000 | PstI | 1x95bp | | 102044 | | 8:15:39 | |
| 64_1.fastq.gz | 4043790 | 1000000 | PstI | 1x95bp | | 89000 | | 7:13:37 | |

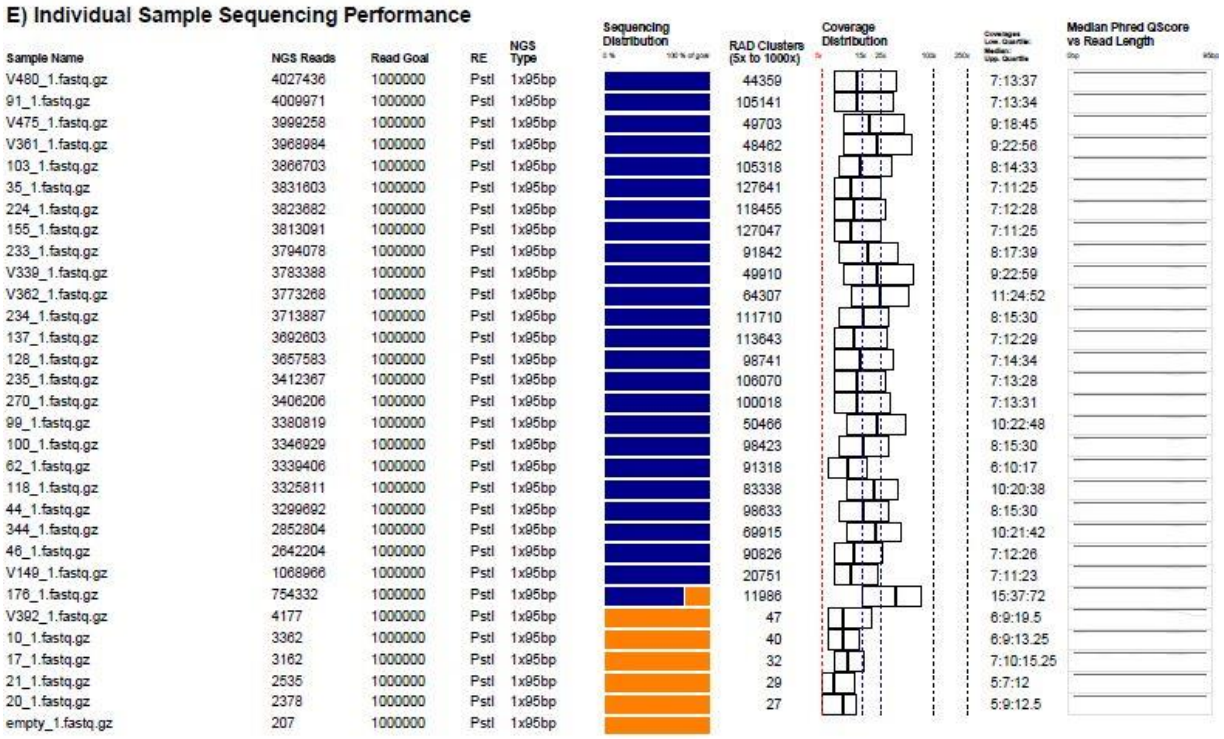



**Figure S. 1** Individual sample sequence performance including number of reads and sequence coverage.

**Table S. 1** Basic information on each linkage group separately for male and female. The map length and average marker spacing are shown in centiMorgan (cM).

| | male | | | | female | | | |
|---|---|---|---|---|---|---|---|---|
| **linkage group** | **No. of markers** | **map length** | **average marker spacing** | **segregation distortion** | **No. of markers** | **map length** | **average marker spacing** | **segregation distortion** |
| 1 | 37 | 64,30 | 1,74 | 45,95% | 39 | 65,0 | 1,7 | 28,21% |
| 2 | 66 | 119,80 | 1,82 | 9,09% | 47 | 121,8 | 2,6 | 19,15% |
| 3 | 40 | 72,90 | 1,82 | 42,50% | 38 | 75,6 | 2,0 | 7,89% |
| 4 | 30 | 85,30 | 2,84 | 23,33% | 41 | 73,4 | 1,8 | 14,63% |
| 5 | 39 | 105,00 | 2,69 | 48,72% | 51 | 95 | 1,9 | 1,96% |
| 6 | 51 | 76,50 | 1,50 | 5,88% | 45 | 65,6 | 1,5 | 60,00% |
| 7 | 15 | 15,50 | 1,03 | 6,67% | 19 | 22,7 | 1,2 | 5,26% |
| 8 | 117 | 116,30 | 0,99 | 47,01% | 56 | 78,5 | 1,4 | 1,79% |
| 9 | 16 | 62,10 | 3,88 | 12,50% | 17 | 68,7 | 4,0 | 5,88% |
| 10 | 30 | 57,20 | 1,91 | 3,33% | 32 | 67,9 | 2,1 | 12,50% |
| 11 | 44 | 64,40 | 1,46 | 86,36% | 40 | 75,3 | 1,9 | 2,50% |
| 12 | 17 | 59,10 | 3,48 | 41,18% | 48 | 75,5 | 1,6 | 8,33% |
| **total** | **502** | **898,40** | **1,79** | - | **473** | **885,00** | **1,87** | - |

# Synopsis

# 6. Synopsis

Klimawandel, Kalamitäten und daraus resultierend vielfach geschwächte Bestände führen zwingend ein Umdenken in der Forstwirtschaft herbei. Vielfach anstehende Verjüngungsplanungen auf großen Schadflächen und Blößen, aber auch der Waldumbau zur Diversifizierung, Stabilisierung und Risikominimierung können nicht allein durch Naturverjüngung umgesetzt werden. Künstliche Bestandesbegründung, Initialpflanzungen auf kleineren Schadflächen und Voranbau sind immer häufiger Mittel der Wahl. Lichtbaumarten, wie die Eiche, eignen sich auf schwachen Standorten, um große Freiflächen wieder aufzuforsten.

Die Ergebnisse dieser Dissertation bilden eine Grundlage zur Entwicklung zukünftiger Anbauempfehlungen, die sich an Waldbesitzer richten und u. a. Hinweise für eine nachhaltige und naturnahe Waldbewirtschaftung mit Blick auf die Anbauwürdigkeit bereits eingeführter Baumarten sowie die Bereitstellung von leistungsstarkem Vermehrungsgut beinhalten. Die Wahl vermeintlich gut geeigneter, zukunfts- und widerstandsfähiger Baumarten sowie deren Herkünfte hat mit Voranschreiten des Klimawandels im Rahmen des Waldumbaus ein steigendes Interesse in der Forstwirtschaft.

Als die slawonische Stieleiche Ende des 19. Jahrhunderts aufgrund schlechter Eichenmastjahre durch den Saatgutimport per Eisenbahn nach Deutschland kam, gab es – anders als heute – noch keine Saatgutzertifizierung. Erst positive Eigenschaften, wie Leistungsstärke und späterer Austrieb (Kapitel 2), lenkten die Aufmerksamkeit in den letzten Jahrzehnten auf diese Stieleichenprovenienz. Für die Erzeugung von zertifiziertem Vermehrungsgut müssen potentiell neue Saatgutbestände in Deutschland eindeutig identifiziert und genetisch charakterisiert werden. Während einige Bestände, wie „Münsterländer Späteiche“, „Späteiche Burg Eltz“ oder „Späteiche Braunschweig“, bereits als Sonderherkünfte ausgewiesen wurden, sind andere Bestände in Deutschland noch weitgehend unbekannt.

Um den Prozess der Identifizierung und genetischen Charakterisierung zu beschleunigen, wurde im Rahmen dieser Dissertation ein Markerset (Kern- und Chloroplastenmarker, Kapitel 3 & 4) aufgestellt. Es kann innerhalb zukünftiger Analysen in der Forstpraxis verwendet werden, um Saatgutproduktionsgebiete zu ermitteln, Vermehrungsgut zu zertifizieren, Mischbestände zu identifizieren und den Genfluss beider Taxa sowie der Bestände späterer Generationen festzustellen. Darüber hinaus eignen sich die in dieser Arbeit verwendeten genetischen Marker um im Rahmen von Plusbaumanalysen die phänotypische Zuordnung abzusichern und gegebenenfalls zu korrigieren. Es besteht erstmals die Möglichkeit den slawonischen Ursprung von Saatgut oder Erntebäumen zweifelsfrei abzusichern.

Um dem Klimawandel allerdings auch langfristig begegnen zu können, müssen wissenschaftliche Erkenntnisse für die Praxis in der Praxis umgesetzt werden. Hier ist das beschriebene

Markerset ein schönes Beispiel. So konnten kurzfristig, über die Firma *ISOGEN*, erfolgreich slawonische Stieleichen für die Saatgutgewinnung identifiziert werden.

## 6.1 Entstandene Einschränkungen und Verbesserungsideen

Nachdem die Ergebnisse der Untersuchungen mit positiven Folgen für die Forstwirtschaft dargelegt wurden, sollen abschließend auch Einschränkungen, Grenzen und Verbesserungsvorschläge zur genetischen Charakterisierung der slawonischen Stieleiche angesprochen werden.

Unter Verwendung des in dieser Arbeit aufgestellten Markersets konnten die genetische Variation der slawonischen Stieleichenbestände in NRW sowie der Fortbestand bzw. die Weitergabe ihrer Variation an die nächste Generation aufgezeigt werden. Allerdings wurden keine slawonischen Bestände in Kroatien, als Referenzbestände, herangezogen. Daher sollte zukünftig auch die geografisch-genetische Variation im Ursprungsgebiet „Slawonien“ charakterisiert und mit der genetischen Variation der in Deutschland charakterisierten Beständen verglichen werden. Ziel dabei ist es, das adaptive Potential dieser Bestände im Vergleich zur Ursprungsregion noch besser einschätzen zu können. Zudem wäre generell eine ausreichende Anzahl von Referenzproben aller slawonischer Stieleichen in Kroatien und Deutschland wichtig, damit die Herkunft einzelner Bäume und Bestände besser abgrenzt werden kann (siehe Kapitel 3). Darüber hinaus empfiehlt es sich auch die Untersuchung zur genetischen Charakerisierung der slawonischen Stieleichenbestände auf andere Bundesländer auszudehnen, um ein besseres Gesamtbild der slawonsischen Stieleiche in Deutschland zu erhalten.

Neben der rein genetischen Charakterisierung, sollten künftig Standortanalysen ergänzend durchgeführt bzw. berücksichtigt werden, da die Standortqualität und dort vor allem der Geländewasserhaushalt aufgrund häufigerer Trockenperioden grundlegend relevant für das Überleben und Wachstum der Pflanzen ist. Darüber hinaus sind Herkunftsversuche sinnvoll, um das Wachstum und die Qualität der slawonischen Stieleiche auf unterschiedlichen Standorten mit der einheimischen Stieleiche vergleichen und daraus Herkunftsempfehlungen ableiten zu können. Die Etablierung von Herkunftsversuchen bildet eine ergänzende Grundlage zu den bereits bestehenden Praxisbeständen (Kapitel 3 & 4) für die erweiterte Bewertung der Anbauwürdigkeit und Anbaueignung der slawonischen Stieleiche.

## 6.2 Schlussfolgerung und Ausblick

Alles in allem liefert die vorliegende Arbeit erste wichtige Ergebnisse zur Etablierung der slawonischen Stieleiche in Deutschland, die weiterhin als Hoffnungsträger im Bezug auf den Klimawandel für deutsche Wälder gilt und im Hinblick des Waldumbaus als nicht ausländische Alternativbaumart zur Diversifizierung und Stabilisierung deutscher Wälder beitragen kann. Der Grundstock für weitere wichtige Analysen, wie beispielsweise die Identifizierung quantitativer Merkmalsloci (QTL), wurde nicht zuletzt durch die Erstellung genetischer Kopplungskarten (Kapitel 5) gelegt. Dabei können Assoziationen zwischen Markern und wichtigen Merkmalen (z.B. später Blattaustrieb, hohe Wuchsleistung) nachgewiesen und diejenigen Genomregionen identifiziert werden, welche an der Kontrolle des jeweiligen Merkmals beteiligt sind. Mithilfe dieser genomweiten genetischen Kartierung lässt sich zukünftig die genetische Basis der slawonischen Stieleiche untersuchen. Praxisempfehlungen zur Etablierung slawonischer Stieleichenbestände können im Hinblick auf die Ertragssteigerung konkretisiert werden.

# Eidesstattliche Erklärung

Hiermit versichere ich, Katrin Schmidt, dass die vorliegende Dissertation mit dem Titel „**Genetische Charakterisierung der slawonischen Stieleiche (*Quercus robur* subsp. *slavonica* (Gáyer) Mátyás) in Deutschland“** selbstständig verfasst und keine anderen als die angegebenen Referenzen, Datenquellen und Hilfsmittel benutzt wurden. Wörtlich oder sinngemäß aus anderen Werken entnommene Stellen habe ich unter Angabe der Quellen kenntlich gemacht. Die Dissertation wurde in keinem anderen Prüfungsverfahren vorgelegt. Weiter erkläre ich, dass ich mich an keiner anderen Hochschule um einen Doktorgrad beworben habe.

Katrin Schmidt
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