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Zmienność genetyczna ekotypów sosny zwyczajnej (*Pinus sylvestris* L.) w Polsce i jej implikacje w badaniach ewolucyjnych i gospodarowaniu zasobami leśnymi w obliczu zmian środowiskowych

Genetic variability of Scots pine (*Pinus sylvestris* L.) ecotypes in Poland and its implications for evolutionary studies and forest resources management in the face of environmental changes

Praca doktorska wykonana
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Spis treści

Lista publikacji stanowiących rozprawę doktorską

Rozprawa doktorska obejmuje wyniki badań przedstawione w trzech artykułach naukowych, z których jeden został opublikowany, a dwa znajdują się w recenzji w czasopismach indeksowanych w Journal Citation Reports. Wyniki badań zostały opisane w trzech następujących artykułach naukowych:

1. **Lasek, M.**, Zaborowska, J., Łabiszak, B., Chmura, D.J., Wachowiak, W. (2024). Genomic Data Support the Revision of Provenance Regions Delimitation for Scots Pine. **Evolutionary Applications**, 17: e70038. DOI:10.1111/eva.70038

   IF_2 =3,2, **IF_5 = 4,1, MNiSW = 140 pkt.**

2. Manuskrypt publikacji**: Lasek M.**, Łabiszak. B., Wachowiak W. „Admixture-Driven Genetic Diversity in Polish Scots Pine Seed Stands: A Blueprint for Climate-Resilient Forestry*”* - **Forest Ecology and Management** – po recenzjach

   IF_2 = 3,7; **IF_5 = 4,1**; **MNiSW = 200 pkt.**

3. Manuskrypt publikacji**: Lasek M**., Walas Ł., Chmura D.J., Wachowiak W. „Balancing Genetic Integrity and Adaptive Potential under Climate Change: The Scots Pine Case ” - **Forest Ecology and Management** – w trakcie recenzji

   IF_2 = 3,7; **IF_5 = 4,1**; **MNiSW = 200 pkt.**

Streszczenie

Zmiany klimatyczne stanowią jedno z najpoważniejszych wyzwań dla współczesnej gospodarki leśnej, wymuszając wdrażanie strategii zarządzania adaptacyjnego. Działania te powinny koncentrować się na wykorzystaniu możliwie najlepiej dostosowanych populacji gatunków drzew leśnych, a w konsekwencji całych ekosystemów wobec dynamicznie zmieniających się warunków środowiskowych. Kluczowym elementem tych strategii jest rozpoznanie i ochrona zasobów genowych drzew leśnych o istotnym znaczeniu ekologicznym i gospodarczym. Utrzymanie ewolucyjnego potencjału adaptacyjnego gatunków w znacznej mierze zależy od posiadanej zmienności genetycznej, dlatego zachowanie możliwie najszerszego jej spektrum jest niezbędne dla zapewnienia ciągłości funkcjonowania ekosystemów leśnych i trwałości gospodarki leśnej. W obliczu pesymistycznych prognoz dotyczących przyszłości lasów zagadnienie to nabiera szczególnego znaczenia.

Niniejsza rozprawa doktorska obejmuje trzy powiązane tematycznie artykuły naukowe, poświęcone sośnie zwyczajnej (*Pinus sylvestris* L.). Jest to kluczowy gatunek złożonych ekosystemów leśnych Eurazji, o ogromnym znaczeniu ekologicznym, gospodarczym i społecznym oraz długiej historii badań podstawowych i stosowanych. Wzorce zmienności neutralnej i adaptacyjnej, historia demograficzna gatunku, a także rola w ekosystemach leśnych są szeroko badane. Na przestrzeni lat podejmowano liczne próby określenia wartości hodowlanej i genetycznej oraz rozpoznania zmienności wewnątrzgatunkowej poprzez wieloletnie badania, w tym również proweniencyjne. Stanowiły one podstawę między innymi do wyróżnienia najcenniejszych drzewostanów sosny, a także wyznaczenia regionów nasiennych. Pomimo tych wieloletnich badań w Polsce nadal brakuje kompleksowej oceny genomicznej zmienności najcenniejszych

populacji sosny zwyczajnej w kontekście obowiązującej regionalizacji nasiennej, której zasadność w świetle aktualnej wiedzy może wymagać istotnej rewizji.

Celem podjętych przeze mnie badań było rozpoznanie struktury genetycznej, zmienności fenotypowej i klimatycznej najcenniejszych polskich drzewostanów sosny zwyczajnej, zarządzanych w ramach ścisłego systemu regionalizacji nasiennej. Badaniami objęto 27 wyłączonych drzewostanów nasiennych (WDN), reprezentujących wszystkie 24 aktualnie obowiązujące regiony nasienne sosny zwyczajnej w Polsce. W badaniach uwzględniono również 31 populacji pochodzących z naturalnego zasięgu gatunku w Europie, w tym z dziesięciu karpackich stanowisk zlokalizowanych w Polsce, które stanowiły grupę referencyjną. W badaniach zastosowano szereg podejść metodologicznych integrujących narzędzia analityczne i dane z zakresu genetyki populacyjnej oraz konserwatorskiej. Uzupełnieniem analiz genetycznych były pomiary cech fenotypowych oraz charakterystyka warunków klimatycznych badanych drzewostanów nasiennych.

Pierwszy artykuł (Lasek i in. 2024; Evolutionary Applications, 17: e70038) koncentruje się na scharakteryzowaniu struktury genetycznej aktualnie obowiązujących regionów nasiennych sosny zwyczajnej w Polsce, reprezentowanych przez najcenniejsze wyłączone drzewostany nasienne oraz wybrane drzewa mateczne. W tym celu wykorzystałam zestaw 16 mikrosatelitarnych markerów jądrowych SSR, 13 polimorficznych markerów mitochondrialnego DNA oraz ponad 37 tysięcy markerów SNP. Stwierdziłam wysokie podobieństwo genetyczne badanych populacji oraz znaczny poziom zróżnicowania wewnątrzpopulacyjnego. Obserwowany wzorzec wskazuje na wspólną historię postglacjalną badanych populacji oraz sugeruje potencjalną admiksję różnych pul genowych w trakcie rekolonizacji. Ponadto wykazałam, iż zróżnicowanie genetyczne badanych drzewostanów nie odzwierciedla ściśle zdefiniowanej regionalizacji nasiennej gatunku w Polsce. Przedstawione wyniki mają zatem istotne znaczenie dla gospodarki leśnej, gdyż stanowią głos w dyskusji na temat rewizji granic regionów nasiennych w Polsce, szczególnie w kontekście niepokojących wyzwań, z którymi obecnie zmaga się sosna zwyczajna w Europie.

W drugiej pracy (Lasek i in., Forest Ecology and Management, po recenzjach) poddaję weryfikacji hipotezę zakładającą wspólne pochodzenie polskich drzewostanów sosny zwyczajnej, wynikające z postglacjalnej historii demograficznej gatunku oraz admiksji różnych pul genowych. Przeprowadziłam badania filogeograficzne, stosując obszerny zestaw danych, obejmujący ponad 37 000 genomowych polimorfizmów pojedynczych nukleotydów (SNP) i 12 markerów mitochondrialnego DNA. Pierwotny materiał badawczy rozszerzono o populacje referencyjne pochodzące z Europejskiego zasięgu, w tym również z Karpat Zachodnich, łącznie analizując 56 stanowisk. Wykazałam, iż historyczne wymieszanie się różnych europejskich linii genetycznych zwiększyło różnorodność genetyczną polskich populacji sosny zwyczajnej, zwiększając ich bogactwo genetyczne i zarazem przypuszczalnie potencjał adaptacyjny. W porównaniu do populacji referencyjnych z europejskiego zasięgu, polskie drzewostany WDN odznaczały się wysokim poziomem zróżnicowania genetycznego i wyższym bogactwem haplotypów wewnątrz populacji. Zastosowane podejście pozwoliło również na określenie unikalnej historii ewolucyjnej południowych populacji górskich w Polsce, odznaczających się odmiennymi pulami genowymi. W pracy przedstawiono również implikacje uzyskanych wyników dla adaptacyjnego leśnictwa w kontekście zmian klimatycznych, podkreślając istotne znaczenie polskich populacji w programach zarządzania zasobami genetycznymi gatunku w Europie.

Trzeci manuskrypt (Lasek i in, *Forest Ecology and Management*, w trakcie recenzji), przedstawia analizy integrujące dane fenotypowe i klimatyczne badanych 27 populacji z wyłączonych drzewostanów nasiennych, uzupełnione o informacje genetyczne oparte na markerach SNP oraz modelowanie wydajności produkcji leśnej w prognozowanych scenariuszach zmian klimatycznych. Uzyskane wyniki wskazują na istotne zróżnicowanie fenotypowe badanych drzewostanów, szczególnie w zakresie cech wzrostowych. Sama zmienność klimatyczna okazała się być niewystarczająca, aby wyjaśnić różnice w efektywności wzrostu, a obserwowane zróżnicowanie fenotypowe nie może być wytłumaczone neutralną strukturą genetyczną. Dla pełniejszego zrozumienia genetycznego podłoża obserwowanej zmienności fenotypowej konieczne są dalsze badania, ukierunkowane na identyfikację genomowych sygnałów lokalnej adaptacji, a także testy potomstwa i doświadczenia z wykorzystaniem wspomaganej migracji.

Wyniki badań wskazują na potrzebę rewizji strategii zarządzania populacjami sosny zwyczajnej, w tym zasad regionalizacji stref nasiennych, oraz podkreślają konieczność wykorzystania materiału rozmnożeniowego o wysokim zróżnicowaniu genetycznym. Takie podejście jest kluczowe dla zachowania różnorodności genetycznej, a tym samym utrzymania długoterminowej produktywności i stabilności drzewostanów sosnowych w szybko mieniającym się środowisku. W świetle postępujących zmian klimatycznych oraz już obserwowanych negatywnych ich skutków, należy rozważyć zastosowanie strategii ukierunkowanych na adaptacje względem tych zmian, takich jak wspomagana migracja czy wspomagany przepływ genów.

Summary

Climate change is one of the most serious challenges for contemporary forest management, necessitating the implementation of adaptive management strategies. Such actions should focus on utilizing populations of tree species that are possibly best adapted, thereby supporting the resilience of entire ecosystems under rapidly changing environmental conditions. A key component of these strategies is the identification and conservation of forest tree genetic resources of great ecological, economic and social importance. Maintaining the evolutionary adaptive potential of species largely depends on their existing genetic variability; therefore, preserving the widest possible spectrum of genetic diversity is essential to ensure the continuity of forest ecosystem functioning and the sustainability of forest management. This issue gains particular importance in the light of pessimistic forecasts regarding the future of forests.

This doctoral dissertation comprises three thematically linked scientific articles focused on Scots pine (*Pinus sylvestris* L.) - a key species in the complex forest ecosystems of Eurasia, of great ecological, economic, and social significance, with a long history of both basic and applied research. Over the years, numerous attempts have been made to assess the breeding and genetic value of Scots pine populations, including the long-term provenance trials. These studies formed the basis for identifying the most valuable pine stands and for establishing seed regions. Despite these longstanding efforts, a comprehensive genomic assessment of Poland's most valuable native Scots pine ecotypes is still lacking. Moreover, in light of recent knowledge, the validity of the provenance region delimitation may require substantial revision.

Therefore, the main aim of my research was to investigate the genetic structure, phenotypic variability, and climatic characteristics of the most valuable Polish Scots pine

stands, managed under the strict provenance region system regime. This study investigates 27 Registered Seed Stands (RSS) populations, representing all 24 currently recognized Scots pine seed regions in Poland. Additionally, 31 populations from the species' natural European range, with ten Carpathian sites in Poland, were included as a reference group. A range of methodological approaches was applied, integrating analytical tools and data from population and conservation genetics. Genetic analyses were complemented by measurements of phenotypic traits and characterization of the climatic conditions of the seed stands.

The first article (Lasek et al., 2024; *Evolutionary Applications*, 17: e70038) focuses on characterizing the genetic structure of the current Polish Scots pine seed regions, represented by the most valuable Reserved Seed Stands and selected plus trees. For this purpose, a set of 16 nuclear SSR microsatellite markers, 13 polymorphic mitochondrial DNA markers, and the PiSy50k SNP array were used. High genetic similarity was observed among the studied populations, along with a substantial level of within-population variation. The observed pattern indicates a shared postglacial history of the populations and suggests potential admixture of different gene pools during recolonization. Moreover, the genetic differentiation of the studied stands did not strictly reflect the currently defined provenance regions delimitation in Poland. These results are therefore significant for forest management, contributing to discussions regarding the revision of provenance regions boundaries, particularly in light of the current challenges that Scots pine is facing in Europe.

The second article (Lasek et al., *Forest Ecology and Management*, after revision) examines whether Polish Scots pine stands share a common origin, shaped by postglacial demographic history and admixture of multiple gene pools. Phylogeographic analyses were conducted using an extensive dataset, including over 37,000 genome-wide SNPs and 12 mitochondrial DNA markers. The primary dataset was expanded with reference populations from across the European range, including the Western Carpathians, and tested 56 sites in total. The analyses demonstrate that historical mixing of European lineages increased the genetic diversity of Polish Scots pine populations, enhancing both their genetic richness and adaptive potential. Compared to European reference populations, Polish RSS stands exhibited high levels of genetic variation and greater within-population haplotype diversity. This approach also allowed the identification of unique evolutionary heritage of Polish highland populations, characterized by distinct genetic composition. The study discusses the implications of these findings for adaptive forestry under climate change, highlighting the importance of Polish populations in European genetic resource management programs.

The third manuscript (Lasek et al., *Forest Ecology and Management*, under review) integrates phenotypic and climatic data from the 27 registered seed stands (RSS), supplemented with genetic information based on thousands of SNP markers and forest productivity modeling under forecasted climate-change scenarios. The results revealed substantial phenotypic variation among the stands, particularly regarding growth traits. Climatic variation alone was insufficient to explain the observed differences in growth performance, and the phenotypic variability could not be explained by neutral genetic structure. Further research is needed to elucidate the genetic basis of the observed phenotypic variation, focusing on identifying genomic signals of local adaptation, as well as progeny trials and assisted migration experiments.

Overall, the results highlight the need to revise management strategies for Scots pine populations, including the principles of provenance regions delineation, and emphasize the necessity of utilizing reproductive material with high genetic diversity. Such an approach is crucial for preserving genetic variation and, consequently, maintaining the long-term productivity and stability of Scots pine forests under rapidly changing environmental conditions. In the face of ongoing climate change and its already observed negative impacts, strategies aimed at facilitating adaptation, such as assisted migration and assisted gene flow, should be considered.

Wprowadzenie

Zmiany klimatyczne stanowią jedno z najpoważniejszych wyzwań dla współczesnej gospodarki leśnej, wpływając na stabilność, produktywność oraz zdolność adaptacyjną ekosystemów leśnych, wymuszają opracowanie strategii ukierunkowanych na zarządzanie adaptacyjne (Bolte i in., 2009; Chakraborty i in., 2024; Fady i in., 2016, 2020). Wysoka zmienność genetyczna stanowi kluczowy czynnik determinujący zdolność adaptacyjną gatunków drzewiastych, a tym samym warunkuje stabilność i funkcjonowanie ekosystemów leśnych wobec zagrożeń związanych z postępującymi zmianami środowiskowymi (Aravanopoulos, 2016). Biorąc pod uwagę znaczne tempo tych zmian (Lindner i in., 2010) (raport ICCP, Lindner 2009), z coraz większym niepokojem spoglądamy na przyszłość lasów. Oczekuje się, że obserwowane zmiany czynników klimatycznych doprowadzą do modyfikacji struktury i funkcjonalności ekosystemów leśnych poprzez zmiany m.in. w strukturze genetycznej, fizjologii, wzorcach wzrostu. Biorąc pod uwagę fakt, że większość lasów jest aktywnie zarządzana, istnieje potrzeba zdefiniowania przyszłych zagrożeń i opracowania strategii dla leśnictwa i hodowli lasu w zmieniającym się środowisku. Taka adaptacyjna gospodarka leśna ma na celu promowanie genotypów o wyższych zdolnościach adaptacyjnych do nowych warunków wynikających ze zmian klimatu.

Zdolność gatunków drzew do radzenia sobie ze zmianami klimatu bazuje na trzech wzajemnie uzupełniających się procesach: adaptacji, migracji i plastyczności fenotypowej. Drzewa pod presją zmian klimatu mogą się do nich aklimatyzować (plastyczność fenotypowa), adaptować (ewolucja), migrować (dyspersja i kolonizacja) lub wymierać (lokalnie lub regionalnie). Ze względu na długi cykl życiowy i czas trwania pokolenia u gatunków drzewiastych, tempo ich adaptacji do szybko zmieniających się warunków klimatycznych może być niewystarczająca. W takiej sytuacji kluczowe znaczenie ma plastyczność fenotypowa, a także działania takie jak wspomagana migracja, które mogą zwiększyć szanse przetrwania populacji.

Sosna zwyczajna (*Pinus sylvestris* L.) jest kluczowym gatunkiem ekosystemów leśnych w Eurazji o ogromnym znaczeniu ekologicznym, gospodarczym i społecznym (Brichta i in., 2023). Jako gatunek pionierski i światłolubny, charakteryzuje się najszerszym zasięgiem występowania spośród rodzaju *Pinus*, rozciągając się w kierunku wschód–zachód na obszarze około 14 tys. km od obszarów górskich Półwyspu Iberyjskiego, przez Kaukaz, góry Ałtaj i Sajańskie, aż po wschodnią Syberię. W kierunku północ-południe natomiast jej zasięg jest znacznie węższy (około 2700 km) i przebiega od północnej części Półwyspu Skandynawskiego do gór Pontyjskich w Azji Mniejszej, przy czym najbardziej na południe wysunięte stanowiska gatunku zlokalizowane są w górach Sierra Nevada w Hiszpanii (Boratyński 1993; Houston Durrant i in., 2016). Tak szeroka amplituda ekologiczna i różnorodność siedliskowa gatunku doprowadziła do wykształcenia znacznej zmienności fenotypowej, odzwierciedlonej w licznych formach i typach morfologicznych, a także w zmienności cech fizjologicznych (Kujala & Savolainen, 2012). Dotychczas opisano ponad 150 typów morfologicznych sosny zwyczajnej, różniących się m.in. terminem zawiązywania pąków, tempem wzrostu, mrozoodpornością, budową szyszek i igieł oraz ogólną wartością hodowlaną (Bruxaux i in., 2024; Carlisle & Brown, 1968; Zajaczkowska i in., 2020). Wieloletnie badania proweniencyjne potwierdzają tę zmienność (Barzdajn i in., 2016; Giertych, 1993; Hall i in., 2021; Hurme i in., 1997), wyróżniając szereg zdefiniowanych ekotypów, do których zalicza się m.in. sosnę masztową, ryską, bolewicką, rychtalską, gubińską, taborską, augustowską i inne.

Zainteresowanie ekotypową zmiennością sosny zwyczajnej sięga początków rozwoju nauk leśnych, gdy w 1824 roku we Francji założono pierwsze doświadczenie proweniencyjne. Na początku XX wieku rozpoczęto serię międzynarodowych badań proweniencyjnych prowadzonych przez konsorcjum naukowców grupy IUFRO (ang. *The International Union of Forest Research Organizations*). Badania typu *common garden* konsekwentnie wykazywały duże różnice w cechach ilościowych i fenotypowych między populacjami pochodzącymi z różnych części zasięgu gatunku (Hurme i in., 1997; Perry i in., 2016; Savolainen & Pyhäjärvi, 2007; Shutyaev i Giertych 1998). Populacje pochodzące z obszarów nizinnych Europy Środkowej z reguły wyraźnie odznaczały się na tle konkurencyjnych proweniencji ogólnoświatowych. Pochodzenia z Polski, Niemiec, Belgii i krajów bałtyckich cechują się szybkim wzrostem, wysoką przeżywalnością, szeroką tolerancją siedliskową oraz dużą odpornością na choroby i szkodniki. Wieloletnie badania prowadzone przez grupę IUFRO przyczyniły się do włączenia najlepszych polskich populacji, takich jak Miłomłyn, Rychtal, Supraśl, Bolewice czy Kubryk, do programów selekcji, ponieważ konsekwentnie osiągały one bardzo dobre wyniki wzrostowe w testach zarówno krajowych jak i międzynarodowych (Barzdajn i in., 2016; Giertych, 1979, 1980; Kowalczyk, 2019; Szeligowski i in., 2023). Z kolei populacje z regionów chłodniejszych (Fennoskandia) wykazują wyższą odporność na mróz i suszę, co czyni je wartościowymi w kontekście adaptacji do zmieniającego się klimatu.

W Polsce sosna zwyczajna jest jednym z najważniejszych gatunków lasotwórczych. Zajmuje 59,1 % powierzchni leśnej kraju, obejmując łącznie ponad 5,5 mln ha, oraz dostarcza około 56 % całkowitej produkcji drewna, co czyni ją kluczowym gatunkiem z punktu widzenia gospodarki leśnej w Polsce (Szmyt, 2025). W obliczu powyższych faktów, szczególnego znaczenia nabiera potrzeba racjonalnego gospodarowania materiałem rozmnożeniowym**.** W obrębie kraju wyznaczono regiony pochodzenia dla głównych gatunków lasotwórczych, obejmujące wysoko cenione w gospodarce leśnej lokalne ekotypy. Zgodnie z wymaganiami UE, państwa członkowskie są zobowiązane do wyznaczenia regionów pochodzenia dla wszystkich gatunków objętych produkcją leśnego materiału rozmnożeniowego (Dyrektywa 1999/105/WE). W przypadku sosny zwyczajnej, regionalizacja nasienna obecnie obejmuje 24 regiony pochodzenia, które określają ramy ścisłego użytkowania i zarządzania leśnym materiału rozmnożeniowym (LMR) i ograniczają użycie materiału spoza danego obszaru (Rozporządzenie Ministra Środowiska z dnia 29 lipca 2015 r., Dziennik Ustaw z dnia 8 września 2015 r., poz. 1328). Celem tych regulacji jest ochrona lokalnych zasobów genetycznych oraz ograniczenie potencjalnie niekorzystnych skutków niekontrolowanego przemieszczania nasion (Matras, 2013). W obrębie regionów pochodzenia źródło materiału rozmnożeniowego zazwyczaj stanowią gospodarcze drzewostany nasienne (GDN) oraz wyłączone drzewostany nasienne (WDN). Wyłączony drzewostan nasienny to drzewostan dojrzały o wysokiej jakości fenotypowej, zdolny do obfitego obradzania nasion, wyłączony od wyrębu i przeznaczony wyłącznie do produkcji leśnego materiału rozmnożeniowego (LMR). Zarówno GDN, jak i WDN mogą obejmować drzewa o wyjątkowych cechach fenotypowych, wybierane do pozyskiwania nasion oraz zakładania plantacji nasiennych. Jednakże w świetle zmian klimatycznych i ich negatywnego wpływu na stabilność, produktywność oraz zdolności adaptacyjne lokalnych populacji, praktyki te mogą wymagać istotnej rewizji lub modyfikacji, co jest obecnie przedmiotem dyskusji wśród badaczy i praktyków (Zubkowicz, 2022). Wiąże się to z pilną potrzebą przeprowadzenia kompleksowych badań integrujących dane genomowe, fenotypowe i środowiskowe w zarządzaniu leśnymi zasobami genetycznymi gatunków drzew. Podejście to jest kluczowe dla identyfikacji mechanizmów lokalnej adaptacji i przewidywania reakcji populacji na zmiany klimatu (Archambeau i in., 2023; Fady i in., 2016, 2020; Feng i in., 2024; Perry i in., 2024).

Historia ewolucyjna europejskich drzew leśnych, w tym sosny zwyczajnej, jest obecnie stosunkowo dobrze poznana (Bennett & Provan, 2008; Hewitt, 2000; Taberlet i in., 1998). Badania filogeograficzne i paleoekologiczne wykazały, iż rozmieszczenie drzew, a tym samym obserwowane wzorce zróżnicowania genetycznego, w dużej mierze wynikają ze zmian demograficznych związanych z kurczeniem się zasięgu w okresie ostatniego maksimum glacjalnego i późniejszych zmian związanych z holoceńską ekspansją postglacjalną (Petit i in., 2003; Tollefsrud i in., 2008). Te historyczne procesy pozostawiły trwałe ślady w genomach gatunków drzew strefy umiarkowanej i borealnej, a prowadzone od ponad czterech dekad badania molekularne pozwoliły na zrekonstruowanie polodowcowych szlaków migracji oraz dynamiki demograficznej populacji. Badania ujawniły między innymi istotną rolę głównych refugiów glacjalnych położonych w południowej Europie (Wahlsteen i in., 2023) w kształtowaniu współczesnych wzorców genetycznych, co przyczyniło się do opracowania powszechnie uznanej koncepcji *'southern richness' versus 'northern purity'* (Heuertz i in., 2004; Tollefsrud i in., 2008). Koncepcja ta zakłada, że szybka kolonizacja z niewielu odizolowanych refugiów pozostawia wyraźny ślad genetyczny, przejawiający się niższym poziomem zmienności w przypadku niedawno skolonizowanych obszarach w porównaniu ze źródłami kolonizacji. Wzorzec ten wynika z postglacjalnych efektów założycielskich i stałego wzrostu dryfu genetycznego wzdłuż szlaków migracyjnych. Koncepcja ta, mimo kontrowersji, jest przekonującym wyjaśnieniem wzorców filogeograficznych wielu gatunków drzewiastych, jednakże nie może w pełni wytłumaczyć geograficznych wzorców zmienności genetycznej sosny zwyczajnej w Europie. Według danych paleobotanicznych, sosna zwyczajna przetrwała nie tylko na południu Europy, ale także w Europie Środkowej - na Węgrzech, Słowacji (Willis & Van Andel, 2004) oraz w Karpatach (Haesaerts i in., 2010), co miało niebagatelny wpływ na kształtowanie współczesnej struktury genetycznej gatunku w Europe. W oparciu o markery mitochondrialnego DNA wykazano wyraźne rozmieszczenie geograficzne mitotypów, odpowiadające głównym obszarom refugialnym w południowej części zasięgu. Oprócz klasycznie opisywanych refugiów południowoeuropejskich coraz więcej danych, opartych głównie na makroszczątkach roślinnych z okresu wczesnego holocenu (Kullman, 2002, 2008) oraz analizach mitochondrialnego DNA (Dering i in., 2017; Żukowska i in., 2023), sugeruje istnienie refugiów zlokalizowanych w północno-wschodniej części Europy. Badania wskazują na istnienie odrębnej linii genetycznej *mt*DNA w rejonie północno-wschodniej Europy i Fennoskandii, odróżniającej się od mitotypów wykazanych w refugiach Półwyspu Iberyjskiego, Apeninów oraz Azji Mniejszej (Naydenov i in., 2007). Niskie bogactwo haplotypów oraz wysoka częstość występowania mitotypu "północnego" w populacjach Fennoskandii i zachodniej Rosji wskazuje, że obszary te mogły stanowić niezależne źródło postglacjalnej ekspansji. Wzorce te zostały potwierdzone również w badaniach jądrowego DNA bazujących na markerach SNP, gdzie dodatkowo wykazano dwutorową kolonizację Fennoskandii oraz mieszanie się różnych linii genetycznych w strefach kontaktu w Europie Środkowej, ukształtowanej w wyniku wielokrotnych fal postglacjalnej rekolonizacji (Łabiszak & Wachowiak, 2024). W badaniach Bruxaux i in. 2024, bazujących na analizie zestawu kilkudziesięciu tysięcy markerów SNP, wykazano niski poziom zróżnicowania populacyjnego (F_{st} = 0,048) w skali całego Euroazjatyckiego zasięgu gatunku. Podobne wyniki uzyskali Wójkiewicz & Wachowiak (2016), wnioskując że Polskie populacje sosny zwyczajnej, obejmujące tereny górskie, torfowiska i niziny, wykazują bardzo wysoki poziom podobieństwa genetycznego. Generalnie, badania neutralnego tła genetycznego Polskich populacji sosny, wskazują na wysoki poziom zmienności wewnątrzpopulacyjnej oraz niski stopień zróżnicowania pomiędzy populacjami. Taki wzór jest po części skutkiem homogenizującego efektu intensywnego przepływu genów za pośrednictwem pyłku a także potencjalnie działalności człowieka.

W Polsce pomimo wieloletnich badań z zakresu filogeografii, genetyki populacyjnej i konserwatorskiej sosny zwyczajnej (Bruxaux i in., 2024; Dering i in., 2017, 2021; Łabiszak i Wachowiak, 2024; Nowakowska i Rakowski, 2005; Nowakowska, 2003; Pyhäjärvi i in., 2020; Tóth i in., 2017; Wójkiewicz & Wachowiak, 2016; Żukowska i in., 2023), rozpoznanie genomowych podstaw zmienności i zróżnicowania najcenniejszych drzewostanów oraz ich pochodzenia nadal pozostają niewystarczająco zbadane. Dotychczasowe badania dostarczyły licznych danych dotyczących historii demograficznej oraz struktury genetycznej sosny (Parducci i in., 2005; Pyhäjärvi i in., 2007; Vila-Cabrera i in., 2011; Żukowska i in., 2023), jednak ograniczenia związane ze schematem próbkowania i rozdzielczością zastosowanych markerów molekularnych uniemożliwiały sformułowanie ogólnych wniosków dotyczących struktury genetycznej drzewostanów reprezentujących wszystkie strefy nasienne gatunku w kraju. Nie integrowały również kompleksowych analiz zmienności genetycznej z oceną fenotypową i środowiskową (Wachowiak i in., 2014; Wójkiewicz i in., 2016). Rozpoznanie zmienności zasobów genetycznych sosny zwyczajnej jest kluczowe dla lepszego przewidywania reakcji populacji na zmieniające się warunki środowiskowe oraz dla opracowania skutecznych strategii ochrony i zarządzania najcenniejszymi populacjami sosny zwyczajnej w Polsce. Jest to szczególnie istotne w kontekście postępujących zmian klimatycznych. Prognozy oparte na modelach zmian klimatycznych wskazują, że zasięg sosny zwyczajnej w Europie może ulec znacznej reorganizacji i redukcji, przy czym najbardziej dotknięte zostaną drzewostany z jego centralnej i południowej części (Dyderski i in., 2025). Aktualnie leśnictwo zmaga się z coraz poważniejszymi skutkami zmian klimatu, takimi jak zwiększona podatność drzew na patogeny i szkodniki, wydłużające się okresy suszy czy rozprzestrzeniająca się jemioła pospolita (*Viscum album ssp. austriacum*), która atakuje całe drzewostany sosnowe (Walas i in., 2022). Wraz z postępującymi zmianami klimatu wymienione czynniki będą wywierać coraz większy negatywny wpływ na przeżywalność i kondycję drzewostanów. Wzrost stresu środowiskowego prawdopodobnie zwiększy niedostosowanie adaptacyjne między obecną kompozycją genomową populacji a tą umożliwiającą skuteczne przystosowanie się do nowych warunków środowiskowych. Ze względu na to że drzewa są organizmami długowiecznymi, ich naturalna zdolność do szybkiej adaptacji jest ograniczona, a najcenniejsze populacje sosny zwyczajnej w Europie są narażone na rosnący stres środowiskowy, który przebiega zbyt szybko, aby naturalny proces lokalnej adaptacji mógł za nimi nadążyć (Aitken i in., 2008). To podkreśla konieczność prowadzenia świadomych działań zarządczych, w tym rewizji i dostosowania stref pochodzenia oraz planowania interwencji wspierających przetrwanie i odporność gatunku. W tym kontekście podjęto próbę przedyskutowania tych kwestii dla jednego z najważniejszych ekonomicznie i ekologicznie gatunków drzew.

W niniejszej pracy, wykorzystanie markerów mitochondrialnego DNA umożliwiło prześledzenie historii filogeograficznej oraz odtworzenie potencjalnych ścieżek migracji gatunku w obrębie środkowoeuropejskiego klinu “północ-południe”. Porównanie warunków klimatycznych i środowiskowych umożliwiło natomiast umiejscowienie populacji lokalnych w szerszym, europejskim kontekście klimatycznym, co może stanowić podstawę do opracowania skuteczniejszych strategii zarządzania leśnymi zasobami genetycznymi i ograniczenia strat związanych ze zmianami klimatu. Regionalizacja nasienna ekonomicznie istotnych gatunków lasotwórczych stanowi fundament nowoczesnej gospodarki leśnej w wielu krajach europejskich, dlatego uzyskane wyniki mają istotne znaczenie aplikacyjne, wspierające podejmowanie decyzji hodowlanych i zarządczych w obliczu rosnącej śmiertelności drzew oraz postępującej utraty ich optimum adaptacyjnego. W konsekwencji praca ta wnosi wkład nie tylko do badań nad różnorodnością genomową kluczowych gatunków drzew leśnych, lecz także do trwającej dyskusji nad kierunkami hodowli i zarządzania lasami w warunkach dynamicznych zmian środowiskowych, oddziałujących na populacje w całym zasięgu występowania sosny zwyczajnej.

Cel i hipotezy

Biorąc pod uwagę kluczowe znaczenie zmienności genetycznej dla funkcjonowania ekosystemów leśnych oraz jej wciąż niedostateczne rozpoznanie, niniejsza praca przedstawia kompleksową charakterystykę zmienności najcenniejszych drzewostanów sosny zwyczajnej w Polsce oraz omawia implikacje uzyskanych wyników dla zarządzania zasobami leśnymi w warunkach postępujących zmian środowiskowych. Celem rozprawy doktorskiej jest rozpoznanie poziomu neutralnej zmienności oraz zróżnicowania genetycznego ekotypów sosny zwyczajnej (*Pinus sylvestris* L.) reprezentujących wszystkie strefy nasienne gatunku w Polsce, a także identyfikacja procesów historycznych, demograficznych i środowiskowych kształtujących współczesną strukturę genetyczną tych populacji. Dotychczas nie prowadzono kompleksowych badań genomowych obejmujących najstarsze i najbardziej wartościowe drzewostany sosny zwyczajnej, porównujących wszystkie regiony nasienne, które stanowią kluczowe zasoby genowe dla gospodarki leśnej. Praca miała również na celu określenie relacji pomiędzy wzorcami zmienności genetycznej a lokalnym gradientem środowiskowym oraz ocenę potencjału adaptacyjnego badanych populacji w kontekście prognozowanych zmian klimatu i możliwych przesunięć zasięgu gatunku.

W niniejszej rozprawie weryfikuję następujące hipotezy:

H1- Hipoteza zmienności ekotypowej

Zmienność fenotypowa ekotypów sosny zwyczajnej ma odzwierciedlenie w zmienności genetycznej i odpowiada przyjętej regionalizacji nasiennej dla tego gatunku.

H2- Hipoteza admiksji populacji

Zmienność genetyczna polskich populacji sosny jest efektem procesów rekolonizacji w okresie po ostatnim zlodowaceniu i mieszania się pul genowych o różnym pochodzeniu.

H3 - Hipoteza środowiskowej zmienności genetycznej

Rozkład zmienności genetycznej ekotypów koreluje ze środowiskiem w wyniku podobnej reakcji populacji na dobór naturalny w gradiencie środowiskowym.

Materiały

Aby zweryfikować postawione hipotezy badawcze, przeanalizowano łącznie 1 269 osobników sosny zwyczajnej, w tym 841 drzew pochodzących z 27 wyłączonych drzewostanów nasiennych, reprezentujących wszystkie 24 obecnie uznawane regiony nasienne w Polsce, oraz 428 osobników referencyjnych z populacji górskich i z naturalnego zasięgu europejskiego. Każdy osobnik został poddany analizom genetycznym z wykorzystaniem trzech komplementarnych systemów markerowych o zróżnicowanej rozdzielczości i trybie dziedziczenia, co umożliwiło wieloaspektową ocenę zmienności genetycznej.

Analizy filogeograficzne przeprowadzono z wykorzystaniem 12 markerów mitochondrialnego DNA (*mt*DNA*: PR5, PR7, PR15, PR19, PR20, PR21, PR24, PR25, PR29, PR30, PR31, PR32*) (Donnelly i in., 2017; Łabiszak i in., 2019; Soranzo i in., 1999), dziedziczonych w linii matecznej poprzez nasiona, co pozwoliło na rekonstrukcję potencjalnych dróg migracji, identyfikację historycznych linii genetycznych oraz ocenę postglacjalnej historii kolonizacji gatunku w Europie Środkowej. Neutralne tło genetyczne populacji analizowano przy użyciu zestawu jądrowych markerów mikrosatelitarnych (*n*SSR: *psyl18, psyl25, psyl36, psyl42, psyl44, psyl57, psyl17, ptTX2146, ptTX3025, ptTX4001, ptTX4011, ptTX8446, pTctg4363, spac11.4, spac12.5*) (Chagné i in., 2004; Elsik i in., 2000; Sebastiani i in., 2012). Mikrosatelity są krótkimi, tandemowo powtórzonymi sekwencjami DNA, które wykazują wysoki poziom polimorfizmu pomiędzy osobnikami (Kelkar et al., 2010). Markery *n*SSR umożliwiły ocenę poziomu zmienności wewnątrz- i międzypopulacyjnej oraz intensywności przepływu genów. Uzupełnieniem analiz były wysokoprzepustowe dane genomowe obejmujące ponad 37 000 polimorfizmów pojedynczych nukleotydów (SNP), uzyskane z wykorzystaniem macierzy PiSy50k SNP array (Kastally i in., 2022). Brookes (1999) zdefiniował SNP jako zmienność sekwencji DNA, polegającą na występowaniu różnicy pojedynczego nukleotydu w określonej pozycji genomu pomiędzy osobnikami w populacji. W niniejszej pracy markery SNP dostarczyły informacji zarówno o neutralnym tle genetycznym, jak i o potencjalnie adaptacyjnym charakterze zmienności genomowej.

Dane genetyczne zostały zintegrowane z informacjami środowiskowymi, obejmującymi dane klimatyczne, siedliskowe oraz szczegółowe pomiary biometryczne drzew z wyłączonych drzewostanów nasiennych, w tym cechy wzrostowe. Takie podejście umożliwiło ocenę zależności pomiędzy zmiennością genetyczną, zróżnicowaniem fenotypowym i warunkami środowiskowymi, a także interpretację uzyskanych wyników w kontekście potencjału adaptacyjnego badanych populacji. Łącznie przeprowadzone analizy dostarczyły nowej, kompleksowej wiedzy na temat zmienności genetycznej drzewostanów sosny zwyczajnej reprezentujących wszystkie strefy nasienne w Polsce oraz ich znaczenia dla długoterminowego zarządzania zasobami leśnymi w obliczu postępujących zmian środowiskowych.

Wyniki

W pierwszej części mojej pracy doktorskiej analizuję i porównuję neutralną strukturę genetyczną populacji z wyłączonych drzewostanów nasiennych reprezentujących wszystkie regiony nasienne wydzielone dla sosny zwyczajnej w Polsce (Lasek i in., 2024). Zrozumienie wzorców neutralnej różnorodności genetycznej ma kluczowe znaczenie w genetyce populacyjnej i biologii ewolucyjnej, ponieważ stanowi podstawę do określenia wpływu procesów demograficznych, przepływu genów i doboru naturalnego. Przepływ genów za pośrednictwem pyłku działa homogenizująco na strukturę populacji i jest na tyle silny, że może zacierać ślady historii demograficznej w loci jądrowych (Bruxaux i in., 2024). Efekt homogenizacji genetycznej spowodowany intensywnym przepływem genów jest wyraźnie widoczny w polskich populacjach sosny zwyczajnej. Analiza przeprowadzona z wykorzystaniem algorytmu STRUCTURE, zarówno w wariancie „admixture" jak i bez, nie wykazała wyraźnego grupowania próbek. Nie zaobserwowałam również spójnego wzorca w statystykach podsumowujących stosowanych do określenia optymalnej liczby klastrów (K); wartości te różniły się jedynie nieznacznie pomiędzy kolejnymi K i nie wykazywały przesunięć pomiędzy podzbiorami. Z punktu widzenia różnicowania genetycznego żadna z badanych populacji nie funkcjonuje jako odrębna jednostka, a dodatkowa struktura populacyjna nie została wykryta.

W oparciu o pełny zestaw danych obejmujący 37 957 SNP, przeprowadzono analizę PCA. Zarówno dla SSR jak i SNP, PCA ujawnia podobny wzorzec punktowej koncentracji osobników, przy jednoczesnym niskim stopniu wariancji nie przekraczającym 0,25% dla SNP i 1,39% dla SSR. Najbardziej genetycznie odrębnymi osobnikami w przypadku SNP były te reprezentujące populacje Międzylesie (Md) i Wichrowo (Wi). Porównanie F_{st} pomiędzy populacjami zarówno dla SSR i SNP ujawniło podobny poziom zróżnicowania. Populacja Md jest identyfikowana jako najsilniej odróżniająca się od pozostałych, choć nadal F_{st} osiąga stosunkowo niską wartość (F_{st}= 0,02). Analiza markerów mitochondrialnych wykazała wysokie zróżnicowanie oraz wyoskie bogactwo haplotypowe we wszystkich badanych populacjach, z wyjątkiem populacji Md. Na podstawie zgromadzonych danych, hipoteza **H1 – zmienności ekotypowej** została odrzucona, ponieważ nie stwierdziłam wyraźnego różnicowania się struktury genetycznej populacji sosny zwyczajnej w obrębie polskich stref nasiennych.

W drugiej części mojej pracy doktorskiej analizuję filogeografię i procesy selekcji, które ukształtowały obecnie obserwowaną strukturę genetyczną polskich populacji sosny zwyczajnej. W tym celu skonfrontowałam polskie populacje WDN z próbami referencyjnymi innych pochodzeń europejskich oraz górskich. Chociaż mitotypy H1 i H2 nie są ze sobą blisko spokrewnione, oba występowały we wszystkich polskich populacjach WDN. Mitotyp H2 dominuje w Fennoskandii, gdzie większość populacji charakteryzuje ten wariant genetyczny lub jedynie niewielka domieszką innych mitotypów. W grupie polskich populacji górskich stwierdziłam kilka unikatowych mitotypów, co wskazuje na historyczną izolację oraz ograniczony przepływ genów w linii matczynej. Populację z Pienińskiego Parku Narodowego charakteryzuje jeden mitotyp (H1), natomiast populacja tatrzańska z Wielkich Korycisk (WK) posiadała mitotyp spotykany jedynie w populacjach ze Szwajcarii i Francji. Najwyższe zróżnicowanie genetyczne, mierzone parami wartości F_{st} dla loci SNP, zaobserwowano między grupami Europy Północnej (NE) i Zachodniej (WE) oraz między polskimi populacjami górskimi (PLM) a Europą Zachodnią (F_{st} = 0,01 w obu przypadkach). Natomiast najniższe zróżnicowanie stwierdzono między polskimi populacjami WDN a Europą Południową

(SE) (F_{st}= 0,001). Mieszanie się kilku europejskich linii genetycznych zwiększyło różnorodność genetyczną i zmniejszyło strukturę populacji w WDN, co potwierdza ich wysoki potencjał wykorzystania jako źródło leśnego materiału rozmnożeniowego. Populacje górskie (PLM) reprezentują unikalne, wysoce zróżnicowane pule genów, charakteryzujące się długotrwałą izolacją. Wyniki sugerują że historyczny i współczesny przepływ genów ukształtował zmienność genetyczną polskich populacji WDN, poprzez korytarze migracyjne Europy Środkowej i Wschodniej, tworząc rodzaj centrum różnorodności genetycznej na obszarze współczesnej Polski. Uzyskane dane wspierają **hipotezę drugą (H2) dotycząca admiksji badanych populacji.**

W trzeciej części mojej rozprawy doktorskiej, przeanalizowałam warunki klimatyczne, dane siedliskowe i biometryczne dla populacji reprezentujących 24 strefy nasienne w Polsce. Założyłam, że słaba presja selekcyjna występująca w ramach względnie jednorodnego gradientu klimatycznego Polski, w połączeniu z lokalną zmiennością klimatu oraz plastycznością fenotypową, leżą u podstaw obserwowanego zróżnicowania fenotypowego między prowenienciami sosny zwyczajnej i wpływają na ich tempo wzrostu w różnych regionach (hipoteza środowiskowej zmienności genetycznej - H3). Aby zweryfikować tę hipotezę, poddałam analizie zależność między wskaźnikiem bonitacji (SI) w wieku 100 lat dla każdego stanowiska a wybranymi zmiennymi klimatycznymi, sprawdzając, czy mogą one bezpośrednio wpływać na produktywność populacji sosny zwyczajnej. W większości badanych drzewostanów sosna zwyczajna była gatunkiem dominującym w górnej warstwie drzewostanu. Jednocześnie wszystkie drzewostany charakteryzowały się złożoną strukturą przestrzenną oraz składem gatunkowym, przy czym sosna w większości przypadków stanowiła główny gatunek. Stwierdziłam znaczne zróżnicowanie w średnicy i wysokości drzew pomiędzy badanymi drzewostanami. Choć porównanie to obejmuje zarówno wpływ czynników środowiskowych, jak i neutralnego tła genetycznego, ogólny zakres uzyskanych wartości wskazuje na istotne różnice produktywności pomiędzy analizowanymi stanowiskami. Ponieważ analizą objęłam wyłącznie drzewa dominujące, a zaobserwowane zróżnicowanie nie było jednoznacznie powiązane z typami siedlisk leśnych, część z tych różnic potencjalnie można przypisać uwarunkowaniom genetycznym. Aby porównać warunki klimatyczne pomiędzy badanymi stanowiskami a całym zasięgiem występowania gatunku wykorzystałam dane z bazy CHELSA (Karger i in., 2017) oraz ClimateEU v.4.63 dla okresu 1991–2020 (Marchi i in., 2020). Tą samą bazę danych wykorzystałam do oszacowania przyszłych warunków klimatycznych dla każdego drzewostanu w trzech scenariuszach: (1) MPI-SSP126 (redukcja emisji CO_2 do poziomu zerowego netto około 2075 roku), (2) MPI-SSP370 (utrzymanie emisji na stałym poziomie do 2050 roku) oraz (3) MPI-SSP585 (potrojenie emisji przed 2075 rokiem) (IPCC 2021).

Stwierdziłam niewiele istotnych zależności pomiędzy cechami strukturalnymi drzewostanów a zmiennymi klimatycznymi; najsilniejsza korelacja wskazywała, że wyższa produktywność siedliska (wyrażona powierzchnią przekroju poprzecznego drzewostanu) była związana z większą dostępnością wody. Analiza głównych składowych (PCA), oparta na zmiennych strukturalnych drzewostanów oraz zmiennych klimatycznych, ujawniła dwie częściowo nakładające się grupy populacji, przy czym dwie pierwsze składowe wyjaśniały łącznie 51% całkowitej zmienności. Pierwsza grupa obejmowała populacje zachodnie i południowo-zachodnie, występujące na stanowiskach o niższym współczynniku kontynentalności (TD) i wyższej średniej rocznej temperaturze (MAT), natomiast druga grupa obejmowała pozostałe populacje. Populacje górskie, takie jak Międzylesie, Lipnica oraz Chełmiec, również skupiały się w tej drugiej grupie, zajmując stanowiska charakteryzujące się krótszym okresem bez przymrozków oraz wyższymi opadami.

Podsumowując, polskie pochodzenia sosny zwyczajnej wykazują znaczące zróżnicowanie fenotypowe, którego nie można wyjaśnić neutralną strukturą genetyczną ani gradientami klimatycznymi. Dynamika wzrostu wydaje się być kształtowana przez sumaryczne oddziaływanie wielu czynników biotycznych i abiotycznych, a także przez potencjalnie adaptacyjne zróżnicowanie genetyczne, którego nie można jednoznacznie ocenić za pomocą neutralnych markerów genetycznych. Ze względu na ograniczoną heterogeniczność klimatyczną w obrębie Polski, ścisłe strefowanie pochodzeń może ograniczać zdolność adaptacyjną populacji w warunkach przyszłych scenariuszy klimatycznych. Na podstawie zebranych danych odrzucam **hipotezę środowiskowej zmienności genetycznej - H3.**

Podsumowanie i wnioski

Niniejsza rozprawa dostarcza nowej wiedzy na temat dostępnych zasobów genomowych oraz relacji genetycznych pomiędzy najcenniejszych ekotypami sosny zwyczajnej. Analiza neutralnego tła genetycznego polskich populacji wykazała, że charakteryzują się one wysokim zróżnicowaniem wewnątrzpopulacyjnym oraz niskim pomiędzy populacjami. Jednocześnie, analizy markerów mitochondrialnego DNA sugerują, że polskie drzewostany stanowią genetyczny rezerwuar różnorodności na tle Europy. Intensywny przepływ genów w obszarze Europy Środkowej zwiększył bogactwo genetyczne populacji, zredukował strukturę genetyczną oraz najprawdopodobniej przyczynił się do wzrostu ich potencjału adaptacyjnego. W przeciwieństwie do WDN-ów, populacje z polskich gór charakteryzują się silnym zróżnicowaniem genetycznym, unikatowymi mitotypami oraz niskim poziomem admiksji, co wskazuje na ich długotrwałą izolację. Ponadto, polskie populacje sosny zwyczajnej wykazują znaczne zróżnicowanie fenotypowe, którego nie można w pełni wyjaśnić jedynie neutralną strukturą genetyczną ani gradientami klimatycznymi. Wydajność wzrostu sosny wydaje się być kształtowana przez złożone oddziaływanie wielu czynników biotycznych i abiotycznych, a także potencjalnie adaptacyjną zmienność genetyczną nieskorelowaną ze zmiennością neutralnych markerów genetycznych. Ze względu na ograniczoną heterogenność klimatyczną w Polsce, restrykcyjny podział regionów pochodzenia może niezamierzenie ograniczać możliwości adaptacyjne populacji w przyszłych scenariuszach klimatycznych. Konieczne są dalsze badania genomowe skoncentrowane na adaptacyjnych SNP, w połączeniu z testami potomstwa i eksperymentami wspomaganej migracji, aby wyjaśnić genetyczne podstawy obserwowanej zmienności fenotypowej.

Ze względu na położenie w przejściowej strefie klimatycznej o relatywnie ciepłych i suchych warunkach, polskie populacje sosny zwyczajnej mogą stanowić cenne źródło leśnego materiału rozmnożeniowego wykorzystywanego w programach wspomaganej migracji. Znaczna plastyczność fenotypowa oraz widoczny potencjał adaptacyjny polskich populacji sosny zwyczajnej pozwalają z umiarkowanym optymizmem patrzeć na ich dalszą rolę w leśnictwie Europy Środkowej w zmieniających się warunkach środowiskowych. Uzyskane wyniki sugerują konieczność rewizji obecnego podziału regionów nasiennych oraz zasad obrotu leśnym materiałem rozmnożeniowym. Dwutorowa strategia zarządzania powinna obejmować wykorzystanie wysoko zróżnicowanych genetycznie źródeł nasion w leśnictwie adaptacyjnym oraz ukierunkowaną ochronę izolowanych populacji górskich. Integracja danych genomowych, testów potomstwa i monitoringu kondycji populacji może wspierać długoterminową produktywność i odporność lasów sosnowych w Europie Środkowej.

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Załączniki

Oświadczenia

Oświadczenie kierującego pracą

Oświadczam, że niniejsza praca została przygotowana pod moim kierunkiem i stwierdzam, że spełnia ona warunki do przedstawienia jej w postępowaniu o nadanie stopnia doktora nauk biologicznych.

Kórnik, 30.12.2025 r.

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podpis

Oświadczenie autora pracy

Świadoma odpowiedzialności prawnej oświadczam, że niniejsza rozprawa doktorska została napisana przeze mnie samodzielnie i nie zawiera treści uzyskanych w sposób niezgodny z obowiązującymi przepisami.

Oświadczam również, że przedstawiona praca nie była wcześniej przedmiotem procedur związanych z uzyskaniem stopnia doktora w innej jednostce.

Oświadczam ponadto, że niniejsza wersja pracy jest identyczna z załączoną wersją elektroniczną.

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Kórnik, 30.12.2025 r.

OŚWIADCZENIE

Oświadczam, że w pracy:

Lasek, M., Zaborowska, J., Łabiszak, B., Chmura, D.J., Wachowiak, W. (2024). *Genomic Data Support the Revision of Provenance Regions Delimitation for Scots Pine.* Evolutionary Applications, 17:e70038. DOI:10.1111/eva.70038 IF$_2$ =3,2, IF$_5$ = 4,1, MNiSW = 140 pkt.

Mój wkład w powstanie tej pracy polegał na udziale w opracowaniu koncepcji i metodyki badań, przeglądzie literatury związanej z analizowanym zagadnieniem, pracach laboratoryjnych, opracowaniu wyników i ich analizie statystycznej, przygotowaniu oryginalnego manuskryptu, wykonaniu korekty manuskryptu artykułu wg uzyskanych recenzji.

Lasek M, Łabiszak. B, Wachowiak W. (2026). *Admixture-Driven Genetic Diversity in Polish Scots Pine Seed Stands: A Blueprint for Climate-Resilient Forestry.* Forest Ecology and Management

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....

Podpis

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Kórnik, 30.12.2025 r.

OŚWIADCZENIE

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Wachowiak

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OŚWIADCZENIE

Oświadczam, że w pracy:

Lasek M., Walas, Ł., Chmura, D., Wachowiak W. – *„Scots Pine Provenances: Balancing Genetic Integrity and Adaptive Potential under Climate Change"*

Mój wkład w powstanie tej pracy polegał na udziale w opracowaniu metodyki badań, wizualizacji i interpretacji uzyskanych wyników oraz udziale w przygotowaniu manuskryptu artykułu.

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OŚWIADCZENIE

Oświadczam, że mój wkład w powstanie pracy:

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polegał na udziale w analizie danych SNP i interpretacji wstępnych wyników.

Julia Zaborowska
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OŚWIADCZENIE

Oświadczam, że w pracy:

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Mój wkład w powstanie tej pracy polegał na udziale w zbiorze materiału, opracowaniu metodyki badań, udziale w przygotowaniu manuskryptu artykułu oraz odpowiedzi na recenzje.

Lasek M., Walas Ł., Chmura D.J., Wachowiak W. (2026). *Balancing Genetic Integrity and Adaptive Potential under Climate Change: The Scots Pine Case.* Forest Ecology and Management

Mój wkład w powstanie tej pracy polegał na udziale w opracowaniu metodyki badań, udziale w interpretacji uzyskanych wyników oraz w przygotowaniu manuskryptu.

Daniel J. Chm
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Oświadczam, że w pracy:

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Lasek M, Łabiszak. B, Wachowiak W. - „*Admixture-Driven Genetic Diversity in Polish Scots Pine Seed Stands: A Blueprint for Climate-Resilient Forestry”* – Forest Ecology and Management

Mój wkład w powstanie tej pracy polegał na analizie i wizualizacji danych oraz udziale w przygotowaniu manuskryptu.

Łabiszak

Rozdział 1

Lasek, M., Zaborowska, J., Łabiszak, B., Chmura, D.J., & Wachowiak, W. (2024). Genomic Data Support the Revision of Provenance Regions Delimitation for Scots Pine. **Evolutionary Applications**, 17: e70038. DOI:10.1111/eva.70038.

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ORIGINAL ARTICLE OPEN ACCESS

Genomic Data Support the Revision of Provenance Regions Delimitation for Scots Pine

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ABSTRACT

Scots pine is a crucial component of ecosystems in Europe and Asia and a major utility species that comprises more than 60% of total forest production in Poland. Despite its importance, the genetic relationships between key conservation and the commercial value of Scots pine ecotypes in Poland remain unclear. To address this problem, we analyzed 27 populations (841 trees) of the most valuable Polish Scots pine ecotypes, including the oldest natural stands in all 24 regions of provenance established for the species in the country. By examining maternally inherited mitochondrial markers, nuclear microsatellite loci, and thousands of SNP markers from a genotyping array, we evaluated the genetic structure between and within them. These multilevel genomic data revealed high genetic similarity and a homogeneous structure in most populations, suggesting a common historical origin and admixture of populations after the postglacial recolonization of Central Europe. This research presents novel data on existing genomic resources among local ecotypes defined within strictly managed Polish regions of provenance, challenging their validity. Formal tests of the progeny of seed stands are needed to check whether the diversity in adaptation and quantitative traits still supports the delineation of provenance regions. In parallel, the health status of selected populations and the viability of seeds from these regions should be monitored to detect early-stage symptoms of their environmental stress. It seems reasonable that periodic shortages of forest reproductive material (FRM) in a given region of provenance could be supplemented with the one from other regions that match their climatic envelope. Together, our results have important implications for the management of native Scots pine stands, particularly elite breeding populations, as they contribute to the discussion of the boundaries of provenance regions and the transfers of FRM that face increasing climate change.

1 | Introduction

In the face of changing climate, forest tree populations experience increasing environmental pressures, including longer drought periods, intense rainfall, more frequent hurricanes, and outbreaks of pests and diseases (Taeger et al. 2013). As a consequence, they are particularly exposed to increased mortality and will need to adapt or likely change their current distribution ranges following the suitable environmental niche for individual species (Buras and Menzel 2019; Dyderski et al. 2018; Kramer et al. 2010; Saltré et al. 2015). Knowledge of the distribution of genetic variation and relationships between natural populations in the range of species is essential for the development of efficient conservation, management, and breeding strategies for forest trees (Aitken et al. 2008). A better understanding of the genetic relationships between breeding ecotypes representing regions of provenance of the species is particularly important considering the predicted environmental changes that will

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affect forest productivity and tree mortality rates worldwide (Hall et al. 2007; Phillips et al. 2009).

Scots pine (*Pinus sylvestris* L.) is a foundation species of various forest ecosystems and one of the most widely distributed and economically important forest trees that is distributed over most of Eurasia. It is a pioneer heliophilic species capable of thriving in various soil types varying in moisture and nutrient content, stretching from coastal regions to altitudes up to 2600 m in mountainous areas (Boratyński 1993; San-Miguel-Ayanz et al. 2016). Due to its extensive ecological range, it exhibits considerable phenotypic variability, manifested in various forms, ecotypes, and physiological traits in various climates and forest ecosystems, resulting in more than a hundred morphological varieties described (Carlisle and Brown 1968; Tóth et al. 2017). Phenotypic differences in traits such as bud flush and bud set, height increment, growth rate, frost hardiness, cones and needle morphology, and general breeding quality are extensive within the species, as demonstrated by numerous population studies, for example (Hurme et al. 1997; Andersson Gull and Fedorkov 2004; Hall et al. 2021; Giertych 1993; Barzdajn, Kowalkowski, and Chmura 2016; Oleksyn et al. 2001). In Poland, Scots pine is one of the main forests-forming and utility species covering more than 5.5 million hectares and represents the largest growing stock (more than 60% of gross wood production) among all other forest tree species.

Earlier studies using various genomic methods provided much data on the demographic history and genetic structure of *P. sylvestris* throughout its wide range of distribution (Bruxaux et al. 2024; Łabiszak and Wachowiak 2023; Żukowska et al. 2023; Kavaliauskas, Danusevičius, and Baliuckas 2022). The studies identified several genetic linages of the species, but found mostly uniform genetic structure in large geographical areas with very little between-population and high within-population variation. Some signatures of significant population divergence were observed in isolated areas of old refugial regions (Pyhäjärvi, Salmela, and Savolainen 2008; Naydenov et al. 2007; Dering et al. 2017, 2021). However, it is unclear how this range-wide pattern translates to genetic relationships of the primary, oldest populations that were used for breeding. Furthermore, genetic data are needed to advance management strategies for stands that have been valued for decades in silviculture and are maintained under strict regime of regions of provenance.

Throughout the years, numerous attempts have been made to determine the genetic value and elucidate the diversity of the most precious pine populations in Poland. Based on morphological variation, growth and productivity traits, but also biochemical studies, many types, ecotypes, or even breeds have been described and valued in forestry due to the unique phenotypic characteristics of a given population (Remlein et al. 2015; Giertych 1997; Staszkiewicz 1993; Przybylski, Matras, and Sułkowska 2015; Hebda and Wachowiak 2019). However, the origin and genetic relationships between those stands could not be well defined based on morphological or anatomical traits including cone or needle biometry as those vary greatly depending, among others, on habitat, latitude, height above sea level, mating processes in the stand, age of the trees and even by location in the crown (Staszkiewicz 1993; Zajączkowska et al. 2020). To evaluate the repeatability of the characteristics of the most famous ecotypes of Scots pine, many international provenance trials have been established over the years to validate their growth performance and survival. The experiments revealed that the populations of species are characterized by a high diversity of phenotypic and quantitative traits (Hurme et al. 1997; Savolainen and Pyhäjärvi 2007; Shutyaev and Giertych 1997; Perry et al. 2016). The results from international IUFRO experiments indicated that Scots pine populations from Poland belong to the most adaptive and well-performing ones exhibiting a better growth compared to those of other parts of the European range of the species (Giertych 1993, 1991; Giertych and Oleksyn 1992).

According to the regulations of the European Union, each country should determine the provenance regions for each tree species subject to production and marketing of forest reproductive material (FRM). These activities ensure that seed regionalization and seedling transfer prevent the negative effects of uncontrolled movement of reforestation material. Regions of provenances are the areas with uniform ecological conditions where stands show similar phenotypic or genetic characters, taking into account altitudinal boundaries (Council of the European Union, 1999 Council Directive 1999/105/EC of 22 December 1999 on the marketing of FRM). In Poland, there are currently 24 regions of provenance of Scots pine that determine the rules for the use and transfer of FRM (Figure S1). According to this delineation, no outside reproductive material is allowed for use in some of the regions of provenance (assigned with the second digit of their signature different than zero, for example, So11, So21, etc. see Table S1 and Figure S1), and detailed deployment rules are given for the other regions (Regulation of Polish Minister of the Environment of July 29, 2015 on the use of FRM outside the region of origin, Journal Laws of September 8, 2015, item 1328). Typically, the basic material within these provenance regions consists of production seed stands (PSS) and registered seed stands (RSS). The latter are excluded from intensive forest management and are intended exclusively for the production of FRM. Both PSS and RSS can include trees with outstanding phenotypic characteristics (plus trees) dedicated to seed harvesting and the establishment of seed orchards. The rationale behind this delineation was mainly to preserve the gene resources of populations in a given area that are presumably adapted to local environments (Fonder, Matras, and Załęski 2007). However, it is largely unknown to what extent this practice may need some review or makeover. This uncertainty emerges from several factors, such as gene exchange in wind-pollinated species, the influence of foreign pollen from outside provenance regions, mostly unknown genetic basis of ecotype variation including plasticity of some phenotypic traits, and most importantly, increasing pressure of environmental changes that will shift adaptive optima of local populations. Furthermore, due to the lack of suitable molecular tools, the genetic relationships between the populations of Scots pine of key importance in Poland remain unclear.

The following research provides multilevel genomic characteristics and an assessment of mutual genetic relationships between Polish Scots pine populations, representing all regions of provenance and some of the oldest stands (average 165 years) that were recognized as valuable ecotypes of superior phenotype or breeding value and were protected to preserve putatively local gene pool of the species. Those most valuable stands of this species in Poland are managed under strict regime of the regions of provenance. A comparative analysis of those old populations was performed on a large set of genetic markers for the species, including mitochondrial DNA markers and polymorphisms in thousands of genomic regions analyzed with the application of microsatellite markers and SNP genotyping technology. The research provides new data on existing genomic resources and genetic relationships between defined ecotypes. As breeding populations and regions of provenance of Scots pine are the foundations of modern forest management in many European countries, our study provides important information on existing genomic resources of species to support management decisions in light of rising tree mortality rates and loss of its adaptive optima. Therefore, the results contribute not only to genomic diversity studies of keystone forest tree species but also to ongoing discussion on their breeding and management strategies following environmental changes that affect many populations throughout the range of species distribution.

2 | Materials and Methods

2.1 | Plant Material and DNA Extraction

Scots pine needles were collected from 27 native populations in Poland representing all regions of provenance (So) for the species in the country (Figure S1). 841 trees included in this study (28–33 from each location) come from strictly managed RSSs or conservation stands and represent the oldest and most valuable breeding material of the species in the country (Figure 1 and Table S1). Several plus trees from the analyzed populations were also included in the study. Genomic DNA was extracted from fresh needles using a Genomic Mini AX Plant kit (A&A Biotechnology, Poland) following the standard manufacturer protocol. The quality of the extracts was evaluated using a BioPhotometer plus (Eppendorf AG, Germany), and the DNA concentration was adjusted to 40 ng/μL.

2.2 | Mitochondrial DNA (*mt*DNA) Variation

A set of 12 mitochondrial markers (*mt*DNA) (PR: 5, 7, 15, 19, 20, 21, 24, 25, 29, 30, 31, 32) and NAD1 intron B/C were genotyped following the SnapShot method described in (Szczepański, Łabiszak, and Wachowiak 2023). The multilocus genotypes of all markers and spatial *mt*DNA haplotypes (mitotypes) were visualized using QGIS v.3.36 (Figure 1). The phylogenetic relationship among haplotypes was examined in POPART v.1.7 (Bandelt, Forster, and Röhl 1999). HaplotypeAnalysis v. 1.05 software (Eliades and Eliades 2009) has been used to calculate the number of haplotypes detected in each population (H), the effective number of haplotypes (Ne), the haplotypic richness (Rh) and the haplotype diversity (Hd) at the population level. Linear regression equations were fitted between the four measures of genetic diversity and the latitudes of population origin in R v. 4.0.3 (R Core Team, 2022). Furthermore, the relationships between populations were visualized based on the unweighted pair group method with the arithmetic mean function (UPGMA) in the software R v.4.0.3 and visualized in FigTree v 1.4.4. The phylogeographic structure was then tested using two measures of population differentiation, including G_{ST} based on haplotype frequency (Nei 1973) and N_{ST} based on haplotype similarity (Lynch and Crease 1990) in PermutCpSSR v.2.0 (Pons and Petit 1995). We performed the Mantel test to analyze genetic and geographic distances between populations using GeneAlex (Peakall and Smouse 2006). Finally, to define populations that are geographically uniform and maximally differentiated from each other, we performed SAMOVA 2.0.

2.3 | Nuclear Microsatellite (*n*SSR) Variation

16 nuclear microsatellite loci (*n*SSR) described previously (Provan et al. 1998; Elsik et al. 2000; Sebastiani et al. 2012; Chagné et al. 2004) were amplified in three multiplex PCRs (Żukowska et al. 2017) using the Qiagen Multiplex PCR Kit (Qiagen, Germany). The PCR reaction contained 5 μL of Qiagen Multiplex Master Mix, 1 μL of Q-Solution, 0.2 μL of primer mix, 1,8 μL of water and 2 μL of DNA template. The multiplexes comprised the following markers: (I) psyl18, psyl25, psyl36, psyl42, psyl44, psyl57; (II) ptTX2146, ptTX3025, ptTX4001, ptTX4011, spac11.4; (III) ptTX8446 psyl17, pTctg4363, spac12.5. The PCR products were fluorescently labeled and along with the internal size standard GeneScan 500 LIZ (Thermo Fisher Scientific, USA), were separated on the Applied Biosystems 3130xl Genetic Analyzer (Thermo Fisher Scientific, USA). Allele sizes (length of fragments) were determined using GeneMapper software v. 4.0 (Thermo Fisher Scientific, USA). Subsequently, the lengths of fragments, representing allele sizes, were established utilizing GeneMapperTM software v. 4.0 (Thermo Fisher Scientific, USA). All size varieties were manually verified and altered. FreeNA software (Chapuis and Estoup 2006) was run to verify the frequency of null alleles, using a maximum likelihood method and employing the expectation maximization algorithm. The basic genetic parameters were calculated using GenAlEx v. 6.5 (Peakall and Smouse 2006) including the mean number of alleles (A), the number of private alleles (AP), and observed heterozygosity (H_O) and expected heterozygosity (H_E). Deviations from Hardy–Weinberg equilibrium (HWE) in all tested populations were evaluated in GENEPOP v. 4.7.5 (Rousset 2008). To estimate the inbreeding coefficients (FIS) and mean rarefied allelic richness (Ar), we employed FSTAT v. 2.9.4. (Goudet 1995, 2002). We also adjusted the FIS values for the occurrence of null alleles with a Bayesian approach executed in the INEst software v. 2.2 (Chybicki and Burczyk 2009). To assess the level of relatedness across our samples and populations, we calculated pairwise kinship estimates using the Queller-Goodnight relatedness measure (Queller and Goodnight 1989) with the related package in R (Pew et al. 2015). If one population had individuals that were more related to each other than those in other populations, it could potentially bias some population structure analyses. Both the SSR and SNP datasets (see below) were used for these calculations. The relatedness measure ranges from −1 to 1, where negative values indicate individuals are less related than the average, 0 indicates no relatedness beyond what is expected by chance, and 1 represents clones or selfing individuals. To deduce genetic relationships among populations and individuals, we implemented the principal component analysis (PCA) and principal coordinate analysis (PCoA) using a matrix of pairwise Nei genetic distances. Furthermore, we have evaluated the genetic correlations between populations applying STRUCTURE

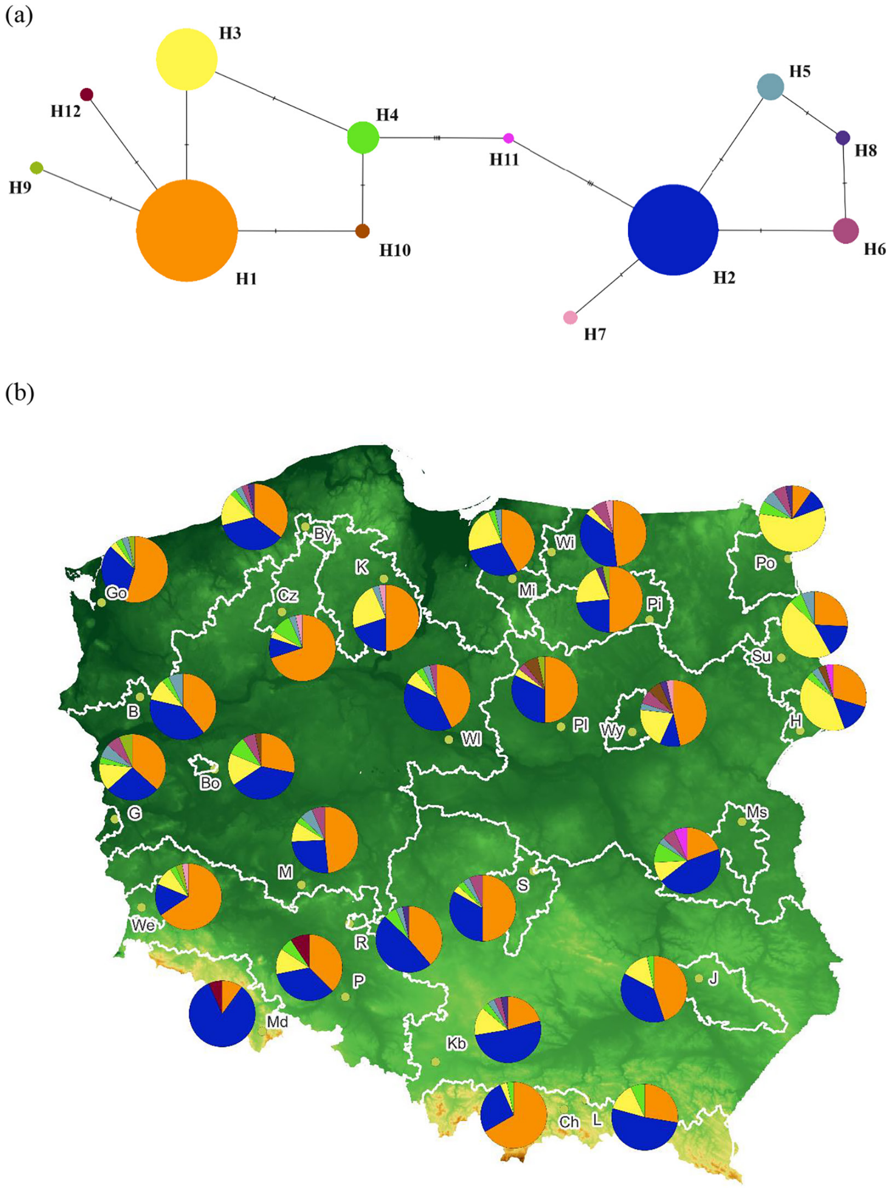

FIGURE 1 | Network of twelve major *mt*DNA haplotypes detected (a) and their spatial distribution in Polish Scots pine populations (b). Boundaries of the analyzed provenance regions are marked with white line based on the data from Polish Forest Seed Office https://www.bnl.gov.pl/. Population abbreviations are presented in Table 1.

v. 2.3.4 (Pritchard, Stephens, and Donnelly 2000) with 500,000 iterations conducted after a burn-in period of 50,000 with 20 independent runs established for the number of genetic groups from K1 to K12. StructureSelector software (Li and Liu 2018) was used to define the optimum K value. Additionally, we have conducted the molecular variation analysis (AMOVA) with 1000 permutations in Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010). Finally, the genetic, environmental, and geographic variables of the analyzed stands were used to assess the genetic structure in our dataset using POPS 1.2 (Jay et al. 2015).

2.4 | Single-Nucleotide Polymorphisms (SNPs) Variation

We genotyped 49,829 SNPs that were used for the development of the PiSy50k SNP array (Affymetrix, Thermo Fisher Scientific, USA). Details about the array design and its validation are described in Kastally et al. (2022). Briefly, the array comprises polymorphisms discovered in exom capture, transcriptome, and candidate genes resequencing in Scots pine. Genotyping was performed in 384-well format on a GeneTitan (Affymetrix, Thermo Fisher Scientific, USA) at Bristol Genomics (UK) after DNA amplification, fragmentation, chip hybridization, single-base extension through DNA ligation, and signal amplification performed according to the Affymetrix Axiom Assay protocol. Genotype calls were obtained using the Axiom Analysis Suite software as recommended by the manufacturer (Applied Biosystems, USA). The initial set of 43,565 SNPs genotyped with the PiSy50k array was examined and filtered to remove low-quality samples and markers. A maximum of 5% of the missing loci per sample was chosen as sample quality. Similarly, SNPs were removed if they were successfully genotyped in less than 5% of the samples, were monomorphic or had a minor allele frequency (MAF) below

1%, or if they showed a significant deviation from the Hardy–Weinberg equilibrium ($p < 0.05$) calculated using PLINK v.1.9 (Purcell et al. 2007). Furthermore, the resulting set of clean data was tested for possible linkage between loci, and one of the SNPs in each linked pair was removed to eliminate disequilibrium in the data set. These LD associations were tested and pruned using PLINK v 1.9 based on squared correlations (argument "–r^2") and its default value of 0.2. Therefore, most of the analyses were performed using the entire SNP data set and LD-pruned SNP datasets. The level of genetic variation present between populations was calculated based on the number of private polymorphisms, the average number of nucleotide differences (K), the expected and observed heterozygosity, and the coefficient of inbreeding in each population using DnaSP v. 6.12.03 and Arlequin. We examined the distribution of the inbreeding statistic (F) across SNP loci within populations as elevated F values would indicate higher inbreeding and thus relatedness between samples. Genetic distances between populations were estimated based on the raw numbers of pairwise differences and calculated in Adegenet v.2.1.9 (Jombart 2008). Furthermore, Mantel isolation by distance (IBD), which estimates the correlation between geographic and genetic distances between populations, was carried out in Adegenet using Edwards distances and 999 permutations. PCA and PCoA were performed to discriminate samples and populations. The population structure and genetic relationships between populations were further evaluated on the average number of pairwise differences, F_{ST} statistics, and the analysis of molecular variance (AMOVA) conducted in Arlequin. To properly handle information obtained from genotyped SNPs, we chose to consider unphased data as multilocus data with unknown gametic phase in all the Arlequin analyses. Furthermore, the samples were grouped following the individual-based Bayesian clustering method, using STRUCTURE v.2.3.4 (Falush, Stephens, and Pritchard 2003). It was launched using Structure_threader v.1.3.0 software (Pina-Martins et al. 2017), which allows for parallel computation and automated run times. Four parallel runs, starting with different seed values, were carried out across the entire SNP data set with two replicates in each run ("-R 2") and other default settings to test how the samples group when split into different numbers of groups of 1 to 30 ("-K 30"). As the runs of the original STRUCTURE program, performed on the full SNP set as well as on the LD-pruned set, appeared overwhelmingly time consuming even when Structure_threader was involved, we decided to prepare 4 subsets of 5000 random SNPs taken from each set and check the coherence of the results across these runs. The sample grouping into up to 30 "populations" was tested; all runs started with 50,000 burn-ins followed by 50,000 MCMC iterations. Mixed and no mixed models were used, with default settings, except that we used correlated allele frequencies with an initial value of α set to 0.04, based on previous inspection. No prior information on the origin of the samples was used, and four replicates ("-R 4") were used for each analysis. To recognize the best number of groups similar to the potential population, we analyze four summary statistics based on the logarithmic probability of the data: mean $\ln p(K)$—averaged over 4 replicates, $\ln'(K)$, $|\ln'(K)'|$ and Delta K (after (Evanno, Regnaut, and Goudet 2005)).

To identify SNPs with allele frequencies significantly deviating from neutrality across the regions of provenances studied and to assess the impact of these SNPs on population structure and differentiation, we performed an outlier detection analysis using two complementary approaches: an F_{ST} outlier scan with OutFLANK v0.2 (Whitlock and Lotterhos 2015) and a population structure-based detection method implemented in the pcadapt v4.3.3 R package (Privé et al. 2020). The use of both methods helps address the inherent challenges of multiple statistical tests, which can increase the probability of detecting and reporting false positive outliers. We set thresholds for the false discovery rate (FDR) at 0.05 and 0.1 using the package R qvalue (Storey, Bass, and Dabney 2022). We visualized the identified outliers for both methods using Manhattan plots and Venn diagrams drawn using ggplot2 and (Wickham 2011, 2016). Since SNPs under local adaptation are likely to be detected by both methods, and recognizing that some outliers may be false positives despite FDR control, we considered only the robust set of loci concordant between both methods as potential adaptive variants. Next, to assess the effects of outlier SNPs on population structure, we performed a PCA on three sets of SNPs: the full set of SNPs, the set excluding outliers, and the set including only outliers. We wanted to explore whether outlier SNPs influence the overall genetic structure among populations. By comparing the result of PCA without outliers vs. with only outliers, we could detect whether the population structure is driven mainly by neutral variation or if it is potentially confounded by loci under selection. Additionally, we performed an admixture analysis using sparse nonnegative matrix factorization in the LEA package using only outliers to formally explore the population structure (Frichot and François 2015). LEA analysis allowed us to identify population clusters and estimate ancestry proportions, providing additional information on the genetic structure shaped by outlier SNPs. We tested ancestral clusters ranging from $K = 1$ to $K = 30$, using 10 replications for each K, to determine cross-entropy. The results were visualized using the POPHELPER Structure Web App v1.0.10 (Francis 2017). Finally, we calculated the pairwise F_{ST} between populations based solely on the outlier SNPs, to quantify the degree of genetic differentiation driven by potentially adaptive loci, as higher pairwise F_{ST} values between populations based on these SNPs would indicate stronger divergence due to selection.

3 | Results

3.1 | *mt*DNA Variation

We used 13 markers, of which 11 were polymorphic, providing 24 haplotypes in 841 trees in the examined stands of Polish Scots pine populations. Of 24, only 12 main haplotypes that occurred more than three times were used to recreate the haplotype network and illustrate their geographic distribution (Figure 1). The final data set consisted of 810 trees with nine polymorphic sites. Overall, the haplotype network revealed two major groups of haplotypes, one including H1 with closely related H3 and several other less frequent haplotypes, and H2 that, together with a few less frequent ones, consisted of a distant haplogroup. The two most frequent haplotypes (H1, H2) were present in every population and in 70% of individual trees, in general. The third most frequent haplotype (H3) occurred in 15% of the trees, was present in 24 of 27 populations, and prevailing in stands from the north-east part of Poland. Although H1 and H2 are not very closely related, both occurred together in all populations

TABLE 1 | Measures of genetic variation at *mt*DNA, *n*SSR and SNPs in the analyzed populations of scots pine.

Population		***mt*DNA**				***n*SSR**							**SNPs**			
(Acronym, RP*)	**N**	**H**	**Ne**	**Rh**	**Hd**	**A**	$\mathbf{A_r}$	$\mathbf{A_p}$	$\mathbf{H_o}$	$\mathbf{H_e}$	$\mathbf{F_{IS}}^{*}$	$\mathbf{F_{IS}}^{**}$	**K**	$\mathbf{H_o}$	$\mathbf{H_e}$	$\mathbf{F_{IS}}^{***}$
Barlinek (B, So30)	30	5	2.961	3.893	0.685	6.750	6.683	0	0.531	0.542	0.073	0.044	10.152	0.327	0.328	0.0012
Bolewice (Bo, So33)	36	6	3.879	4.822	0.766	7.375	6.939	4	0.521	0.540	0.049	0.028	10.104	0.326	0.327	0.0020
Bytów (By, So11)	31	7	3.546	5.484	0.742	6.938	6.825	0	0.575	0.572	0.012	0.016	10.224	0.327	0.327	0.0001
Chełmiec (Ch, So80)	32	4	1.931	2.800	0.499	7.375	7.153	1	0.558	0.558	0.046	0.021	10.345	0.327	0.328	0.0025
Czarne (Cz, So31)	30	6	1.948	4.700	0.503	7.000	6.937	0	0.515	0.541	0.065	0.042	10.247	0.326	0.327	0.0017
Goleniów (Go, So40)	31	6	2.445	4.484	0.611	7.188	7.048	2	0.589	0.570	−0.017	0.006	9799	0.327	0.327	−0.0001
Gubin (G, So34)	32	7	4.206	5.879	0.789	7.438	7.223	1	0.531	0.542	0.035	0.024	9703	0.327	0.327	−0.0013
Hajnówka (H, So23)	30	7	3.556	6.000	0.746	7.063	6.999	1	0.528	0.553	0.062	0.024	10342	0.329	0.328	−0.0015
Janów L. (J, So62)	30	4	2.739	2.931	0.658	7.000	6.933	0	0.535	0.546	0.017	0.013	10.611	0.328	0.327	−0.0025
Kaliska (K, So32)	30	5	2.885	3.800	0.676	6.688	6.629	1	0.535	0.548	0.040	0.041	9957	0.330	0.328	−0.0038
Kobiór (KB, So60)	30	7	2.993	5.724	0.69	6.938	6.877	3	0.546	0.570	0.059	0.014	9950	0.327	0.327	−0.0014
Lipnica (L, So80)	30	4	2.722	2.998	0.655	6.750	6.685	2	0.535	0.546	0.036	0.021	10.233	0.325	0.327	0.0067
Międzylesie (Md, So70)	33	3	1.411	1.993	0.301	6.875	6.643	1	0.549	0.561	0.036	0.022	9787	0.326	0.325	−0.0031
Międzyrzec (Ms, So42)	32	7	3.710	5.843	0.755	7.000	6.872	3	0.498	0.527	0.071	0.028	10.277	0.327	0.327	0.0005
Milicz (M, So30)	32	6	3.130	4.844	0.703	7.063	6.890	1	0.568	0.557	−0.004	0.011	9537	0.328	0.328	−0.0032
Miłomłyn (Mi, So12)	30	5	3.193	3.742	0.71	7.000	6.881	0	0.542	0.544	0.020	0.017	10.728	0.329	0.329	0.0014
Pisz-Dziadki (Pi, So21)	30	5	2.885	3.800	0.676	7.750	7.654	4	0.553	0.543	−0.001	0.208	10.067	0.328	0.328	0.0010
Płońsk (Pl, So40)	30	6	2.761	4.893	0.661	7.438	7.360	0	0.547	0.549	0.020	0.011	10.455	0.327	0.328	0.0022
Pomorze (Po, So20)	32	7	2.707	5.830	0.652	6.938	6.751	0	0.560	0.534	−0.033	0.006	10.275	0.327	0.327	−0.0010
Prószków (P, So50)	32	5	3.483	3.978	0.736	6.938	6.776	1	0.553	0.557	0.024	0.013	10.018	0.328	0.327	−0.0008
Rychtal (R, So52)	34	5	2.563	3.729	0.63	7.125	6.853	1	0.529	0.534	0.024	0.014	10.464	0.329	0.329	−0.0020
Spała (S, So61)	31	6	2.711	4.693	0.653	7.063	6.914	0	0.548	0.535	−0.008	0.007	10.273	0.327	0.327	−0.0008
Supraśl (Su, So24)	31	5	3.280	3.974	0.718	7.500	7.347	2	0.530	0.545	0.044	0.023	10.390	0.327	0.327	0.0017
Węgliniec (We, So51)	32	6	2.142	4.529	0.55	7.500	7.268	0	0.525	0.541	0.045	0.014	9742	0.327	0.326	−0.0014
Wichrowo (Wi, So20)	30	5	2.651	4.000	0.647	6.938	6.866	1	0.556	0.554	0.012	0.010	10.388	0.328	0.327	−0.0024

(Continues)

TABLE 1 | (Continued)

Population (Acronym, RP*)	mtDNA					nSSR							SNPs			
	N	H	Ne	Rh	Hd	A	A_r	A_p	H_o	H_e	F_{IS}*	F_{IS}**	K	H_o	H_e	F_{IS}***
Włocławek (Wł, So30)	30	6	2.882	4.893	0.677	7.375	7.302	2	0.513	0.549	0.083	0.032	9614	0.327	0.327	−0.0004
Wyszków (Wy, So41)	30	8	3.571	6.686	0.745	7.188	7.110	0	0.517	0.529	0.040	0.016	10.429	0.327	0.327	0.0006
Mean	**31**	**5.7**	**2.922**	**4.479**	**0.661**	**7.118**	**6.978**	**1.1**	**0.540**	**0.548**	**0.033**	**0.018**	**10.152**	**0.327**	**0.327**	**−0.0002**

Abbreviations: A, mean number of alleles; A_r, allelic richness; Ap, number of private alleles; F_{IS}*, fixation index calculated with Fstat; F_{IS}**, fixation index calculated with INEST; F_{IS}***, inbreeding coefficient averaged over SNPs; H, number of haplotypes; Hd, haplotype diversity; He, expected heterozygosity; Ho, observed heterozygosity; K, average number of nucleotide difference; N, number of samples; Ne, effective number of haplotypes; Rh, haplotypic richness; RP, Regions of provenance; bold values indicate mean of basic statitistic across all populations.

(Figure 1). Most of the populations studied were characterized by a high haplotypes richness (Table 1). The mean haplotype diversity for all samples was Hd = 0.661; the lowest average haplotype diversity was detected in the Międzylesie population (Hd = 0.301), while the highest average haplotype diversity was detected in Gubin, Bolewice, and Międzyrzec (0.789, 0.766, and 0.755, respectively; Table 1). There was no significant relationship between the measures of genetic diversity and the geographical distribution of the populations. Analysis of genetic relationships between haplotypes using UPGMA revealed a division between two groups as in the case of the haplotype network (Figure S2). The lack of phylogeographic structure was observed in the Mantel test ($r^2 = 0.0012$, $p = 0.350$) and the comparisons of N_{ST} and G_{ST} ($N_{ST} = 0.0372$, $G_{ST} = 0.0293$) (Figure S3). SAMOVA analysis revealed 3 groups for K3 with low differentiation level. The first two included only two populations from southern Poland (group 1 = KB, group 2 = Md), while the third group consisted of the remaining 25 populations (Figure S4).

3.2 | *n*SSR Variation

The final data set consisted of 16 polymorphic loci with mean frequency of null alleles in all populations. Null = 0.009 (range, 0.003–0.014) that did not exceed the cut-off point of Null = 0.19 (Chapuis and Estoup 2006). The highest average number of alleles and alleles richness was observed in Pisz ($A = 7.75$, $A_r = 7.65$) from the northeastern Poland (provenance region So21) and the lowest in Kaliska ($A = 6.69$) from northern Poland (So32) and Międzylesie ($A_r = 6.64$) from southwestern Poland (So70), respectively (Table 1). Only a few private alleles were present in some populations. The observed heterozygosity was at a comparable level in all populations and ranged from 0.498 to 0.575, with an average of 0.540 (Table 1). No deviation from HWE equilibrium was found in the populations, and the F_{IS} index was relatively low or negative. Results of relatedness analysis showed that average kinship between individuals within population is substantially low (mean values of Queller-Goodnight relatedness measure were close to 0), and consistent across all populations. There was no qualitative difference when considering the SSR or SNP dataset and we show result based on SNPs only (Figure 2). No single population showed excess of highly related individuals and thus no individual was removed from further population structure analysis. PCA and PCoA analysis reveal a uniform distribution of genetic variation in Polish Scots pine populations (Figure 3a and Figure S5a). No signatures of the population structure were indicated in Bayesian clustering with STRUCTURE (Figure S6). Genetic differentiation between the populations was generally low; based on the F_{ST} values the most divergent was the population of Międzylesie (Figure 4a). The global F_{ST} conducted in FreeNA was equal to 0.0028 and 0.0023 with and without null alleles, respectively. POPS 1.2 results were fully consistent with STRUCTURE analysis indicating homogeneous genetic structure of examined Scots pine populations.

3.3 | SNPs Variation

All genotyped samples passed the quality threshold and were used in the analyses. From the initial set of 43,565 genotyped

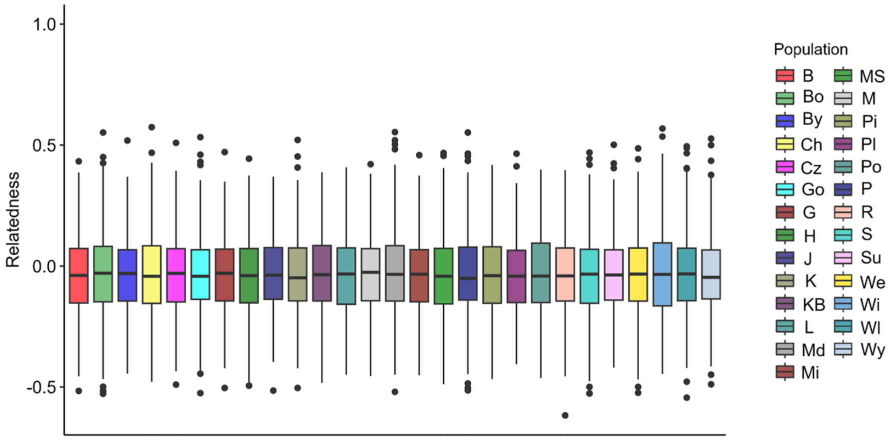

FIGURE 2 | Boxplots showing mean relatedness among individuals across 27 studied pine populations based on all SNPs.

SNPs, 5968 loci were filtered as they exceeded our missing data cutoff level, appeared monomorphic in this sample set, had MAF < 1% or significantly deviated from HWE. The 37,597 resulting markers were considered a "complete" set of SNPs. In this set, 44,688 potential LD pairs were found that involved a total of 26,013 unique loci. After strict pruning of the linked loci, the new set, hereafter "LD pruned," contained 11,583 markers. Both the "whole" and "LD-pruned" SNP sets were used in parallel in subsequent analysis. The populations showed very similar patterns of genetic variation at two sets of SNPs, and here we summarize only the results obtained from the whole set. High within-population genetic diversity and low between-population genetic variation were observed across populations. No fixed or private SNP alleles were found in any population. Nucleotide differences between samples within populations ranged from 9537 (Milicz) to 10,728 (Miłomłyn), with a mean over stands of 10,152 (Table 1). However, when all samples were treated as a group, this average distance dropped to 1624. In each population, about 98.96% of the loci appeared heterozygous. The observed and expected heterozygosity estimates were very similar, and the population-specific values hardly deviated from their mean values (0.327 (SD 0.001) the same for both). There was no indication of inbreeding in any population. The average F_{IS} statistic over loci was −0.0002 (Table 1), and the median values of inbreeding statistic across the populations were close to 0 (Figure S7). No significant correlation was found between genetic and geographic distances ($r^2 = -0.161$, p-value = 0.927) (Figure S8). Close genetic relationships between populations, with exception of most individuals from Międzylesie (Md, So70) and Wichrowo (Wi, So20) were observed in the PCA and PCoA analyses (Figure 3b and Figure S9). In general, genetic differentiation between Scots pine populations was found to be very low, $F_{ST} = 0.0017$ (Figure 4b). The most differentiated pair of populations were Międzylesie and Pomorze sites ($F_{ST} = 0.0075$); however, the LD-pruned set indicated Międzylesie and Wichrowo populations as the least similar in terms of allele frequencies ($F_{ST} = 0.0080$). The low differentiation observed between populations was confirmed by AMOVA, which showed that the gross variation (more than 99%) observed in the Polish population of Scots pine segregates within individual genomes (Table S2). Along with this, the only significant fixation index in the analysis was the inbreeding coefficient of an individual relative to the total population—F_{IT} (Table S3). STRUCTURE analysis showed that the populations defined here based on the sampling locations are all mixtures of many different genetic clusters, and that there was a large variation between runs in the way the samples were assigned. In general, no clear clustering of samples was identified in the "admixture" as well as the "no admixture" version of the STRUCTURE algorithm. No pattern was observed in the summary statistics used for the selection of the best K; they differed weakly between successive K values and were not "phased" between subsets (Figure S10). In terms of genetic distinction, none of the sampling populations exists as a discrete unit, and no other structure was identified.

Using our complete dataset of 37,957 SNPs, we identified a total of 96 outlier SNPs using the pcadapt method and 4 outlier SNPs using OutFLANK (Figure S11). In particular, the number of outlier SNPs detected by pcadapt varied with the FDR threshold: at a more stringent q-value of 0.05, only 48 SNPs were identified as outliers. However, the number of outliers detected by OutFLANK remained consistent regardless of the FDR threshold. Surprisingly, only a single SNP was identified as an outlier by both pcadapt and OutFLANK, highlighting the distinct sensitivities and specificities of these two methods. All the 99 outlier SNPs constituted 0.26% of the total SNP dataset, indicating a minimal overall impact on population structure. This was further corroborated by PCA, where the inclusion or exclusion of these outliers had only a slight effect on the overall pattern observed. Specifically, when outliers were excluded, there was a modest increase in variance explained by the first principal component (PC1), from 0.27% to 2.03%. Despite this, most of the individuals of all populations still clustered closely in the PCA, forming a central group. The only notable deviation was the consistent positioning of individuals in the Md population, which remained distinct regardless of whether outliers were included in the analysis (Figure S9). However, the explained variance was significantly higher when looking at PCA based only on outlier SNPs (PC1 = 16.09%, PC2 = 7.16%), and there was split into three groups: individuals from different populations were mixed between these groups without a clear population-specific or regions of provenance-specific pattern. This suggests that outlier SNPs capture little variation that differentiates individuals. However, those individuals do not cluster to the particular predefined population or regions of provenance categories.

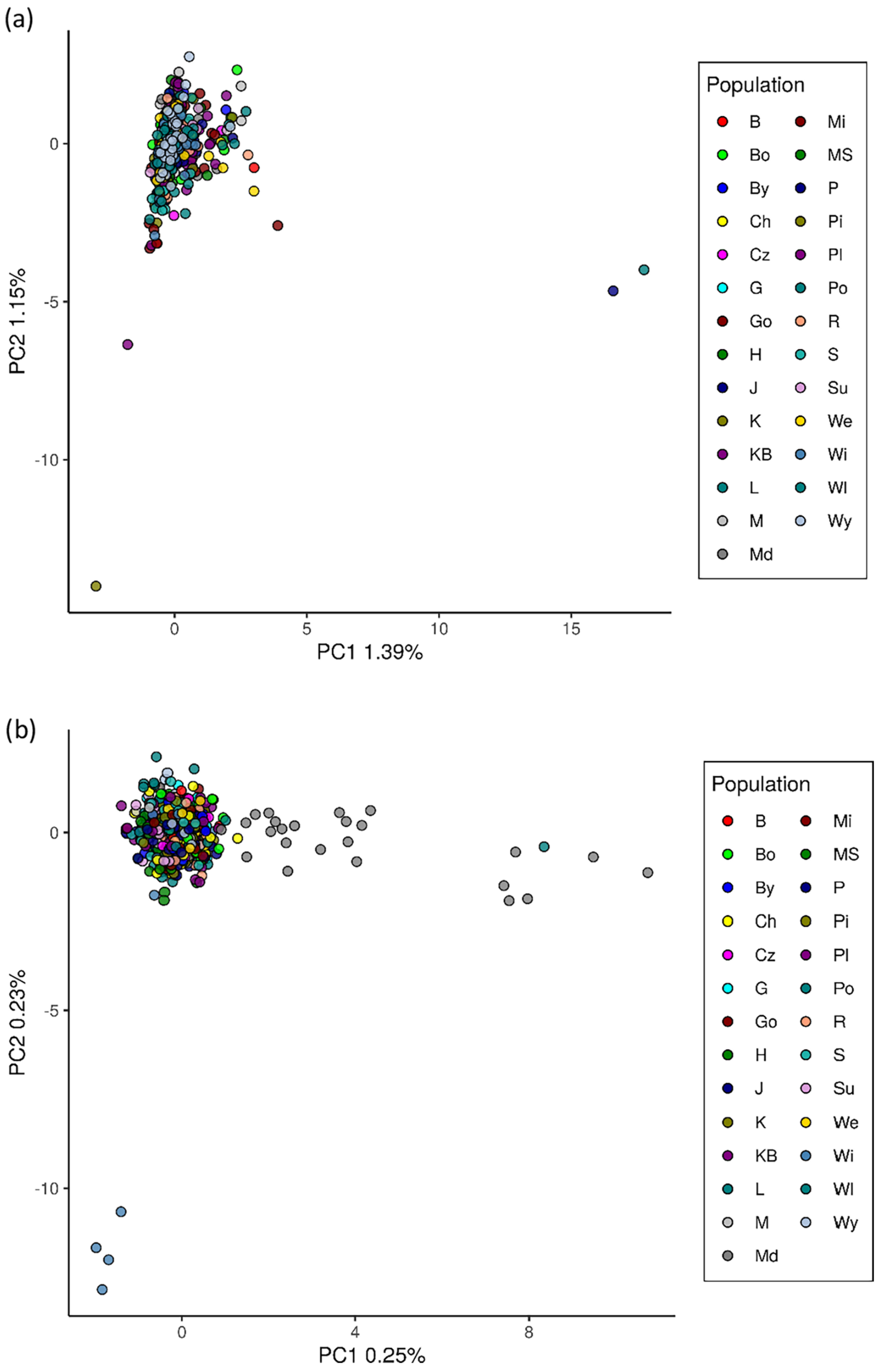

FIGURE 3 | Principal Component Analysis at *n*SSR loci (a) and all SNP loci (b) showing relationships between studied individuals. Most outlier individuals at SNPs loci were from population Md and Wi.

This was also evident when considering the results of the LEA analysis, where the cross entropy decreased sharply at $K=1$ and reached its minimum at $K=15$. These findings suggest that outlier SNPs do not differentiate effectively between populations, since when $K=15$ no distinct population-specific clusters were observed; instead, individuals of various ancestry were present in all populations, albeit in differing proportions. Differentiation between populations based on outlier SNP pairwise F_{ST} was an order of magnitude higher than when all SNPs were used, but still quite weak, with maximum less than 2% differentiation (Figure S12). The F_{ST} values obtained from the SSR markers were comparable to those derived from the SNP outliers, both showing a maximum F_{ST} of 0.02 and producing similar heatmaps (Figure 4c).

4 | Discussion

4.1 | Genetic Relationships Between Populations

In this study, we genotyped and analyzed the distribution of polymorphisms in thousands of genetic markers in a large panel of more than 800 individuals derived from 27 of the most valuable phenotypically and ecologically diverged populations

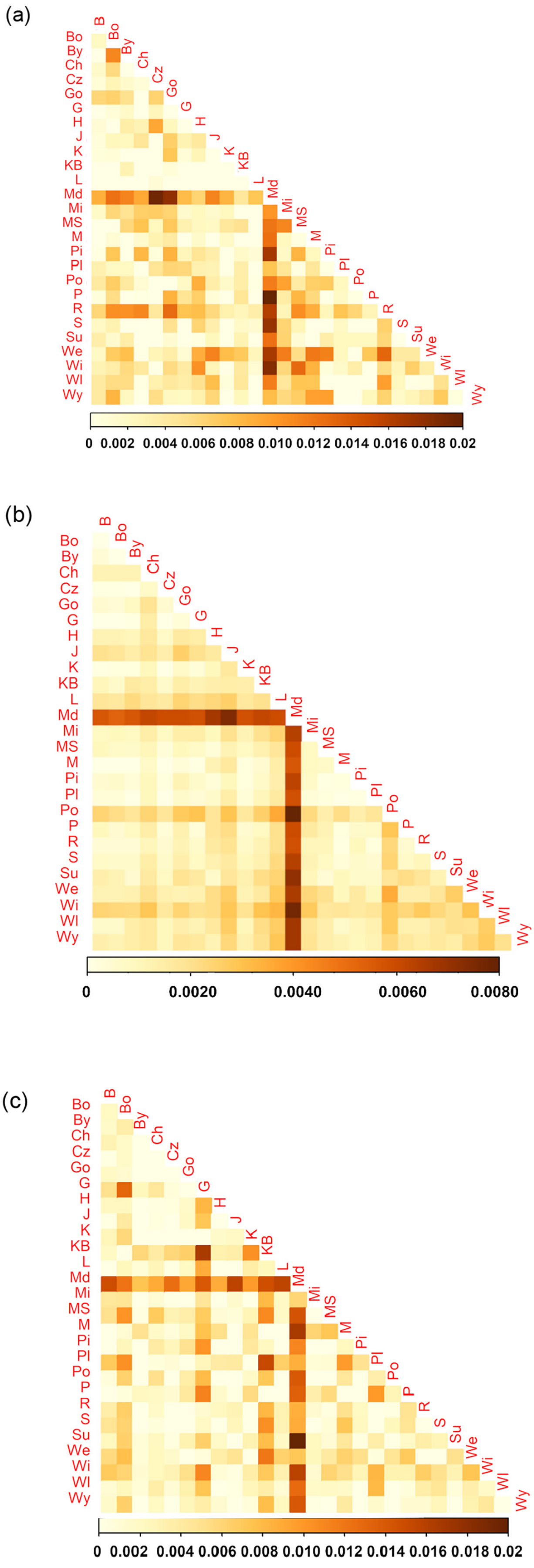

FIGURE 4 | Legend on next page.

FIGURE 4 | Heat map of F_{ST} values between analyzed populations of Scots pine for *n*SSR (a) SNPs (b) and outlier SNPs (c). Order of populations as in Table 1.

of Scots pine in Poland. Genetic variation based on molecular markers derived from genomes of different modes of transmission and inheritance in pines was compared between populations representing different stands and all regions of provenance defined for the species in the country. Proper management of the genetic resources of Scots pine, which is one of the most economically important species for timber production in many European countries, is challenging given the ongoing climate changes and the increased mortality in the range of species. In general, Scots pine populations from Central Europe, including the area analyzed in Poland, demonstrated fast growth rate, high wood quality, fitness, and breeding value on many provenance trails conducted both locally in Poland and also in Eurasia and North America, for example (Barzdajn, Kowalkowski, and Chmura 2016; Giertych and Oleksyn 1992; Stephan and Liesebach 1996). Specifically, the results indicated a large variation in the growth characteristics of Scots pine at the level of a given provenance (Giertych 1993; Przybylski, Matras, and Sułkowska 2015; Szeligowski et al. 2016; Oleksyn and Rachwał 1994).

Until now, most of the genetic studies of Scots pine in Poland refer to the application of a limited number of stands and genetic markers (Wójkiewicz, Litkowiec, and Wachowiak 2016; Be 2016; Wachowiak et al. 2014). However, the sampling and resolution of the markers were usually too low to draw any general conclusions about the genetic relationships between native ecotypes. Furthermore, the regions of provenance of the species were never fully represented in population genetics and genomic studies. Our data show striking genetic similarity at background genetic variation between populations suggesting their common population history. Taking into account their age, they were initiated at the time when different parts of the analyzed territory were, due to the historical geopolitical situation, under the Austro-Hungarian, Prussian, and Russian administrations. Active forest management was in operation at that time, with varying intensity among these different administrative regions. It cannot be excluded that some Scots pine seeds were possibly traded, but perhaps at much smaller scale compared to Norway spruce, due to high availability of local seed sources (Jansen, Konrad, and Geburek 2017; Wachowiak et al. 2024). Therefore, it seems unlikely that analyzed pine populations were established as a result of historical seed transfer from some common sources. Considering the high genetic similarity among the populations studied, they most likely represent natural stands in the area that was recolonized over the last several thousand years.

Mitochondrial DNA, due to the limited ability of seed transfer, allows one to identify fine-scale differentiation between populations compared to pollen-mediated genomes. Gene flow by pollen has a homogenizing effect on population structure and is strong enough to obscure ancestral history in nuclear loci (Bruxaux et al. 2024). Mitochondrial DNA analysis indicated that two main mitotypes (H1 and H2) belong to distinct genetic groups that were fixed in 70% of all individuals. Haplotype H1 corresponds to the main European mitotype present in most southern and western populations, while H2 corresponds to the main Fennoscandian haplotype that is abundant in Finnish stands (Wachowiak et al. 2023). The third most common mitotype, H3, was present in 24 of the 27 populations studied and 15% of all trees analyzed and was dominated by populations from north-eastern Poland. The remaining haplotypes were much less frequent. In general, most of the populations studied were characterized by high haplotype richness, and the results coincide with previous reports showing the highest *mt*DNA in mid-latitude regions (Wachowiak et al. 2023). The results suggest that during the postglacial recolonization process populations of different origins admixed in the central European distribution of the species and newly established Scots pine forests did not diverge significantly, resulting in populations that share a common history. The hypothesis of admixture for the origin of Polish populations is further supported by the results of genetic variation at neutral loci, including SSR and SNPs data. Here, all sampled populations were classified as a single genetic group in STRUCTURE and POPS analyses and showed a similar level of genetic variation. Comparable genetic relationships between most samples were also observed in the PCA analysis. The level of differentiation between populations at the SSR loci (average $F_{ST} = 0.002$) and SNP markers ($F_{ST} = 0.002$) was marginal and lower compared to some earlier estimates in the populations of Polish Scots pine at the SSR loci ($F_{ST} = \sim 0.03$, Hebda and Wachowiak 2019). The most recent study based on a wide range of Scots pines analyzed on nuclear SNP markers revealed relatively low variation between populations ($F_{ST} = 0.048$, Bruxaux et al. 2024). In our study, outlier SNPs did not show any signatures of population differentiation that would correspond to the predefined regions of provenance. Other studies show that there is little correlation between local adaptation and genetic data (Hall et al. 2021; Tyrmi et al. 2020) which does not align with the high morphological diversity observed due to possibly non-genetic origin of some traits variation and adaptation linked to many genes with small individual effects. Certainly, the populations included in previous Scots pine studies did not refer to the oldest stands of the species or were established from open pollinated seeds from those stands, potentially affected by gene flow from much younger commercial stands of unknown origin. Collectively, the results indicate a very uniform genetic background of the most valuable Polish Scots pine populations that were most likely established through gene exchange in large geographical areas that led to high genetic diversity within individual stands and no significant differentiation between populations.

Due to the effectively non-existing population structure in our dataset, a similar pattern of population differentiation observed at SSR and SNP markers does not reflect a higher resolution of the SNP array in genetic diversity and fine-scale population studies (Zimmerman, Aldridge, and Oyler-McCance 2020). In our recent investigations, the SNP array showed much better performance in delineating populations and hybrids of closely related pine species compared to SSR markers (Łabiszak et al. in preparation). In general, both mitochondrial markers and nuclear DNA SNPs are useful for population delineation. The first set of markers that is transmitted maternally without sexual recombination can mark distinct genetic linages and therefore are suitable for phylogeographic studies, while the nuclear markers can additionally indicate targets of natural selection. Our results support the hypothesis of a common origin of the populations and do not provide an indication of a population structure in the studied area, in contrast to that recently described for the range of other parts of the species distribution (Łabiszak and Wachowiak 2023). More recent studies indicated several genetic linages of species that intermixed at some parts of the species range during recolonization but maintained their distinct genetic characteristics despite presumably intensive gene flow between populations over large geographical areas (Łabiszak and Wachowiak 2023; Dering et al. 2017). Significant divergence between populations was also observed in southern refugial regions (Pyhäjärvi, Salmela, and Savolainen 2008; Dering et al. 2021). In wind-pollinated tree species with possible long-distance pollen dispersion events, without strong geographical barriers at regional scale and quite uniform environmental conditions, we should expect a shallow genetic structure and similar genetic background. However, such a fine-scale structure could possibly be maintained if there are some phenological differences between populations, or selection against maladapted alleles would affect population fitness. Due to many shared haplotypes between populations, only two stands from southern Poland (Międzylesie, Kobiór) were indicated as genetically distinct in the SAMOVA analysis. Furthermore, the population of Międzylesie (Md, So70) in the Kłodzko Valley surrounded by the mountains was characterized by the lowest number of mitotypes and mitotype diversity, the low number of SSR alleles and their diversity, as well as one of the lowest estimates of heterozygosity based on SNPs, and was consistently found to be the outlier at the nuclear loci in the PCA and PCoA analyses. Although the non-native origin of that population cannot be excluded, the geographic region where it is located was previously shown to have unique genetic characteristics in other tree species (e.g., *Pinus mugo* (Żukowska and Wachowiak 2017)) and recent evidence indicates the possible presence of a small isolated glacial refugium nearby, which could ensure the persistence of some genetic preglacial remnants in the region (Suchan, Malicki, and Ronikier 2019).

4.2 | Forest Management Implications

Our results have important implications for forest management, as they contribute to the discussion of the boundaries of the provenance regions and the transfers of FRM. The populations analyzed in the study were derived from the 24 regions of provenance defined for Scots pine in Poland that are managed under strict seed transfer regimes. Consequently, the reproductive material derived from a given seed stand should be planted within a given region of provenance, and there are restrictions on the movement of the plant material between the regions. These rules are especially strict for some regions of provenance, which are considered sources of the most valuable populations of Scots pine in Poland. Similar rules were also in operation in previous seed regionalization regulations, where most of these regions were considered “maternal” versus the others, which were considered ordinary regions (Załęski et al. 1994). However, no formal tests were conducted to compare the silvicultural properties of the reproductive material derived from these two types of regions. Some provenance tests in which such a comparison is possible do not prove the superiority of one versus another in terms of individual tree growth and stand productivity (Chmura, Guzicka, and Rożkowski 2021). Furthermore, in these tests, local populations are often among the best for tree diameter or height, but usually not for area-based productivity (Chmura, Guzicka, and Rożkowski 2021; Hebda, Skrzyszewski, and Wachowiak 2017; Hebda, Wachowiak, and Skrzyszewski 2017). However, to date, there has been no coherent comparative assessment of the silvicultural properties of local populations representing different provenance regions. Therefore, although the current delimitation of the provenance regions for Scots pine in Poland is less restrictive than the previous one (Załęski et al. 2000), the ongoing effort to formally test the progeny of seed stands (Sabor et al. 2004) should provide information on whether the diversity in adaptation and quantitative traits still supports this distinction of provenance regions. Such quantitative genetic assessments and progeny tests of the provenance regions combined with environmental and climatic data of the sites would be needed to validate whether the phenotypic differences between the provenance regions are due to local adaptive divergence or plastic responses. Once validated, the application of genomic data related to population history assessments and their signature of adaptation should facilitate decisions on delimitation of provenance regions and support further expansion of its current boundaries into larger areas. Additionally, the condition of selected populations and the viability of seeds from the most valuable provenance regions should be systematically monitored to detect early-stage symptoms of their environmental stress. The results of genetic differentiation at the molecular level provided by our study do not appear to justify strict rules for seed transfer. Moreover, these rules may be counterproductive in terms of assisted migration in the face of ongoing climate changes (Aitken and Bemmels 2016). It seems reasonable that periodic shortages of reproductive material in a given area could be supplemented with FRM from regions of provenance that match their climatic envelope. Such an approach may be inevitable in the near future, as ongoing and projected environmental changes will significantly affect the fitness and productivity of seed stands, influence suitable climatic niches of forest tree species, and their geographical distributions are likely to follow (Dyderski et al. 2018; Chakraborty et al. 2021). The stress associated with droughts, forest fires, catastrophic winds, insect infestations, and mistletoe outbreaks that are already observed in many European forests, including Scots pine (Taeger et al. 2013), will likely intensify in the future, affecting pine populations. For species with a long generation time, such as Scots pine, the adaptive capacity of their populations is based on standing genetic variation inherited over generations rather than spontaneously arising new mutations (Barrett and Schluter 2008). Therefore, adaptive responses of populations in the near future will depend on the already available genetic variation segregating in the populations. A high level of genetic diversity is crucial to the survival of species because it determines their performance, protects against the detrimental effects of inbreeding, and improves adaptive evolutionary potential and phenotypic plasticity (Alberto et al. 2013). Our data show not only the uniform genetic background of Polish Scots pine, but also indicate that the vast majority (> 99%) of its high genetic diversity is present within each population studied. This pattern is likely related to the common history of populations and is maintained by highly efficient pollen and seed dispersal in this random-mating and wind-pollinated species, and its generally large effective population size characterizing forest tree populations (Petit and Hampe 2006). Other genomic studies supported an expansion of current provenance regions of Belgian populations of black alder (*Alnus glutinosa*) into larger seed zones (De Kort et al. 2014). However, in our dataset, the most distinct Md population represents southern stands and pines from the mountain regions of Poland are known to be genetically distinct as compared to lowland stands (Wachowiak et al. 2024). Therefore, we recommend treating this population as a distinct provenance region (So70) and FRM should not be mixed with lowland stands.

The climate niche scenarios for the future distribution of Scots pine (Dyderski et al. 2018; Chakraborty et al. 2021) are particularly unfavorable for forests in Poland, where Scots pine is the main tree species. Increased environmental stress will likely broaden the genomic offset between the current genomic composition and the one needed to cope with novel environmental conditions. This will likely negatively affect the fitness of individual trees and whole populations, significantly affecting forest production. Taking this into account, it seems that the most valuable Scots pine in Europe is exposed to increasing environmental stress, occurring at a pace too fast to be followed by local adaptation (Aitken et al. 2008). Taking into account the changing environments, soon the local ecotypes of the species may not be suitable any more at the place of their occurrence. If these scenarios are confirmed, the present-day regionalization of basic forest material may not hold in the near future. Together, considering existing genomic resources and the uniform distribution of standing genetic variation between populations, our data contribute to the discussion of the revision of existing provenance regions of the species. Similar investigations should also be carried out on other species and in other countries considering that provenance regions are delimited for many forest tree species subject to production and marketing of FRM.

5 | Conclusions

The research took advantage of the latest genomic resources and developments in Scots pine genomic studies to assess the level and distribution of genetic variation in mitochondrial and nuclear genomes in the most valuable populations of the species in Poland. The study provides new knowledge on the existing genomic resources and the genetic relationships of the ecotypes of the species. The data indicate high genetic similarity between the analyzed populations, suggesting their common history resulting from postglacial recolonization and admixture of populations of different origins. Considering the uniform genetic background of populations and the increasing pressure of environmental changes that globally have a negative impact on the condition and survival of forest tree ecosystems, it appears that the current delineation of provenance regions of Scots pine may need to be revised. Our results do not justify either strict seed transfer rules. We suggest a formal test of the progeny of seed stands to check whether diversity in adaptation and quantitative traits still supports the distinction of particular provenance regions. In parallel, the condition of selected populations including their health status, symptoms of environmental stress (e.g., drought or pest damage), flowering, fructification, and the viability of seeds from the most valuable provenance regions should be systematically monitored to detect early stage symptoms of their environmental stress. It seems reasonable that periodic shortages of reproductive material in a given area could be supplemented with FRM from provenance regions that match their climatic envelope. Together, we believe that this case study provides useful data in the discussion of existing breeding and forest management strategies in Scots pine to counteract the negative consequences of environmental changes. This is consistent with the ongoing efforts and long-term strategy of the Polish State Forests. Taking into account the similar regionalization practice of FRM in many European countries, the research addresses questions of forest management of general importance in forestry.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All genomic data used in this manuscript are available at the Dryad Digital repository: https://doi.org/10.5061/dryad.0cfxpnw9q.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Material

Suppl. Table S1. Characteristics of the analysed populations of Scots pine included in the study

Population	Acronym	Region of provenance	Age	Latitude	Longitude	Elevation (m a.s.l)
Barlinek	B	So30	188	52.93	15.181	101
Bolewice	Bo	So33	150	52.375	16.13	103
Bytów	By	So11	146	54.224	17.29	121
Chełmiec	Ch	So80	125	49.642	20.589	532
Czarne_Czl.	Cz	So31	154	53.582	16.986	146
Goleniów	Go	So10	176	53.65	14.695	18
Gubin	G	So34	199	51.981	14.863	71
Hajnówka	H	So23	171	52.619	23.603	180
Janów_Lub.	J	So62	124	50.709	22.31	224
Kaliska	K	So32	215	53.833	18.293	125
Kobiór	KB	So60	186	50.032	18.95	273
Lipnica	L	So80	140	49.722	20.87	503
Międzylesie	Md	So70	171	50.287	16.742	547
Międzyrzec	MS	So42	144	51.965	22.865	164
Milicz	M	So30	155	51.458	17.239	198
Miłomłyn	Mi	So12	162	53.828	19.934	147
Pisz	Pi	So21	158	53.519	21.679	136
Płońsk	Pl	So40	110	52.701	20.551	131
Pomorze	Po	So20	151	53.983	23.451	150
Prószków	P	So50	188	50.559	17.804	208
Rychtal	R	So52	166	51.185	17.957	217
Spała	S	So61	204	51.568	20.203	201
Supraśl	Su	So24	155	53.232	23.362	176
Węgliniec	We	So51	188	51.285	15.2	201
Wichrowo	Wi	So20	182	54.03	20.43	110
Włocławek	Wl	So30	188	52.602	19.119	69
Wyszków	Wy	So41	184	52.661	21.467	129

Suppl. Table S2. AMOVA analysis based on genetic variation among 27 populations of Scots pine

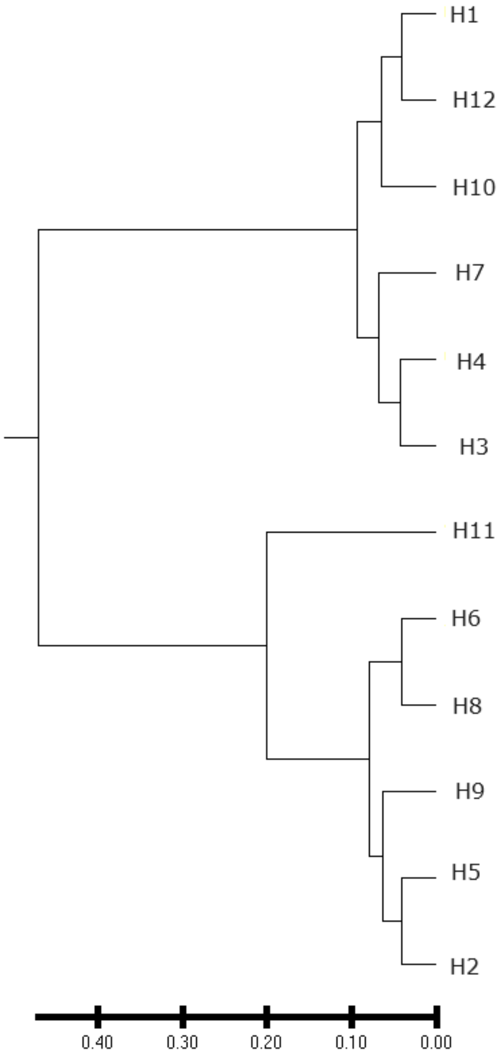
Set		Source of variation	Sum of squares	Variance components	Percentage variation
27 populations	**Whole**	**Among populations**	175091.4	10.36	0.169
		Among individuals within populations	4792847.5	-1.19	-0.019
		Within individuals	4959829	6114.14	99.850
		Total	9927767.9	6123.31	100
	LD-pruned	**Among populations**	44759.3	2.57	0.164
		Among individuals within populations	1228928.9	-0.17	-0.011
		Within individuals	1271531	1567.29	99.85
		Total	2545219.1	1569.69	100

Suppl. Table S3. Fixation indices and significance test results from AMOVA analysis, showing amounts of heterozygosity at various levels of population structure

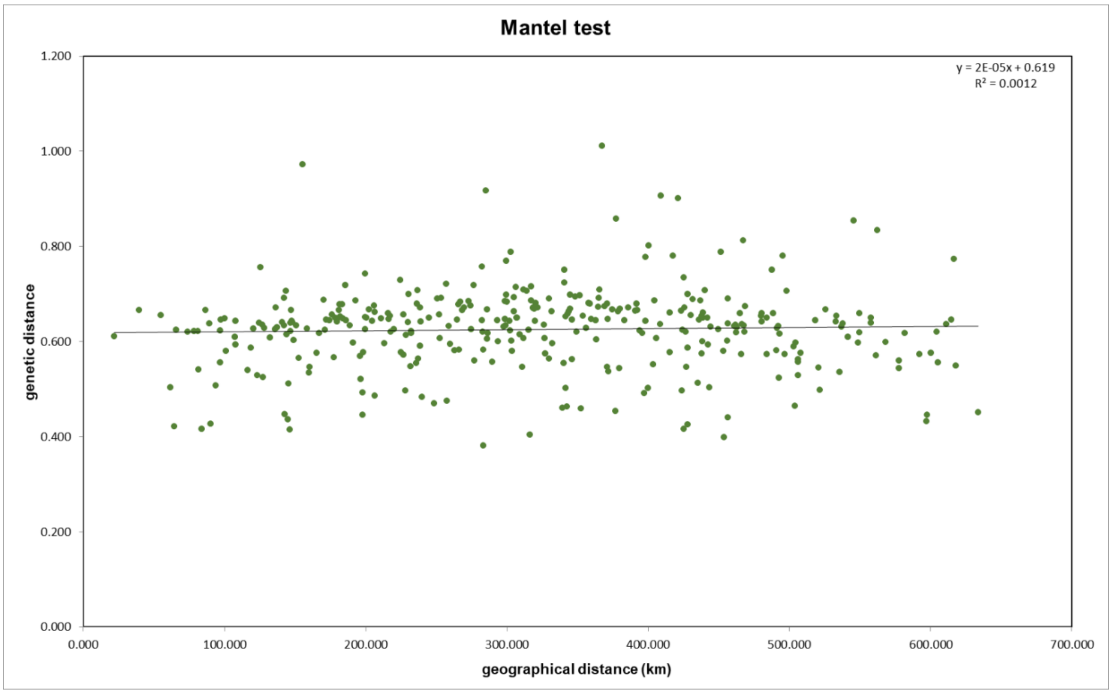
SNP set	Fixation index	Value	*p*-value
Whole	**FIS**	-0.00020	0.809
	FST	0.00169	1.000
	FIT	0.00150	0.000
LD-pruned	**FIS**	-0.00011	0.608
	FST	0.00164	1.000
	FIT	0.00153	0.000

Suppl. Figure S1. Regions of provenance for Scots pine in Poland (source: Journal of Laws of September 21, 2015, item 1425. Regulation of the Polish Minister of the Environment of July 29, 2015 on the list, areas and maps of regions of origin of forest reproductive material)

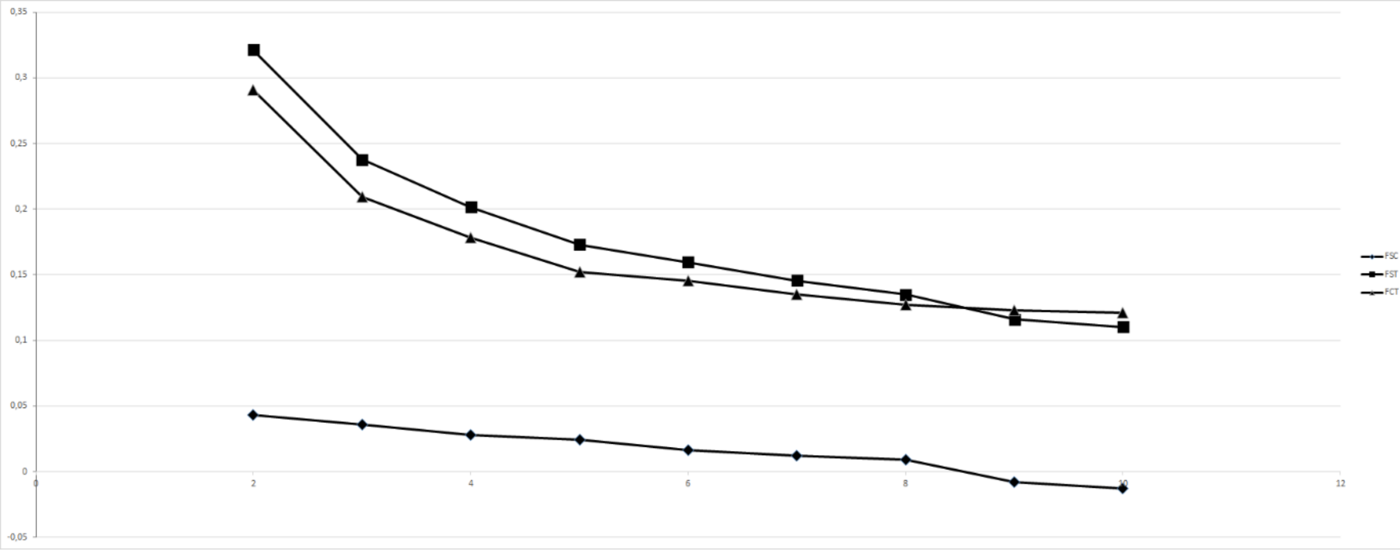
Suppl. Figure S2. UPGMA tree for 12 main haplotypes occurring in 27 Scots pine populations



Suppl. Figure S3. Mantel test based on the matrix of genetic distance at *mt*DNA markers and geographical distances between populations

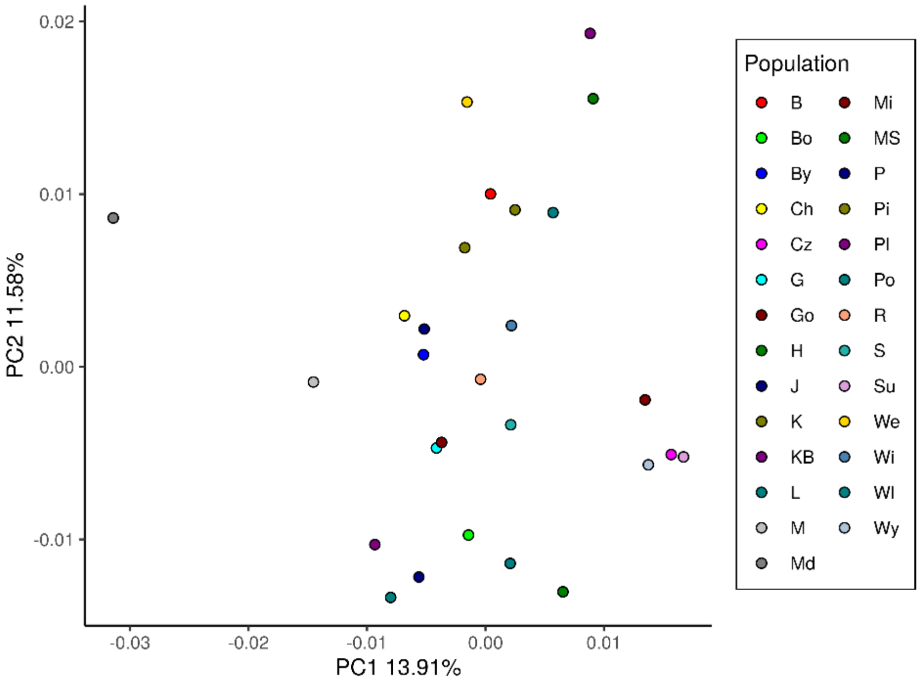


Suppl. Figure S4. SAMOVA analysis for K3 ravels 3 genetic groups. Group1 = "KB”, Group2 ="Md", Group3 = 25 remaining populations

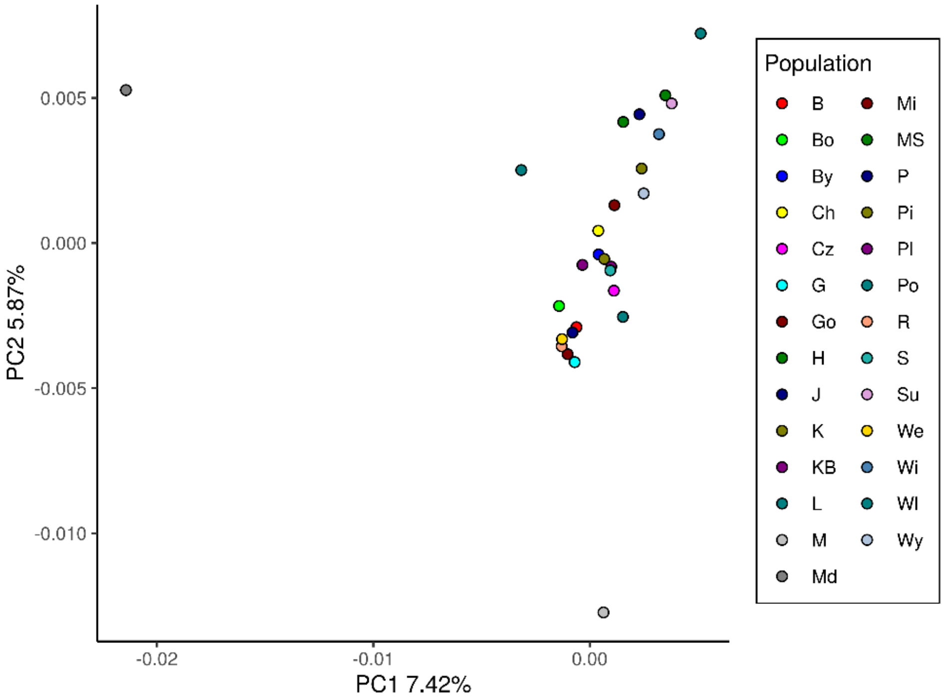


Suppl. Figure S5. Principal Coordinate Analysis (PCoA) showing relationships between studied populations at (a) SSR loci and (b) SNPs markers

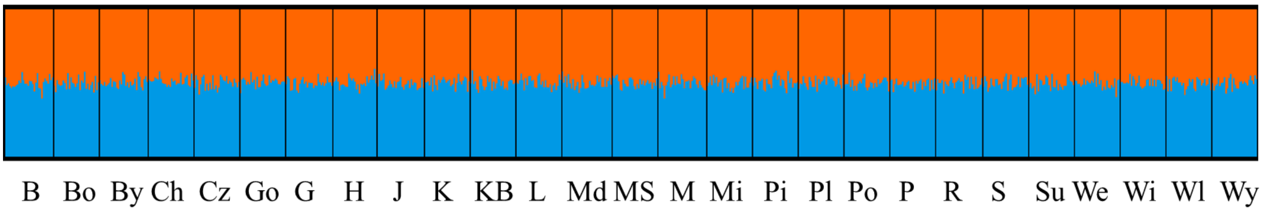
(a)



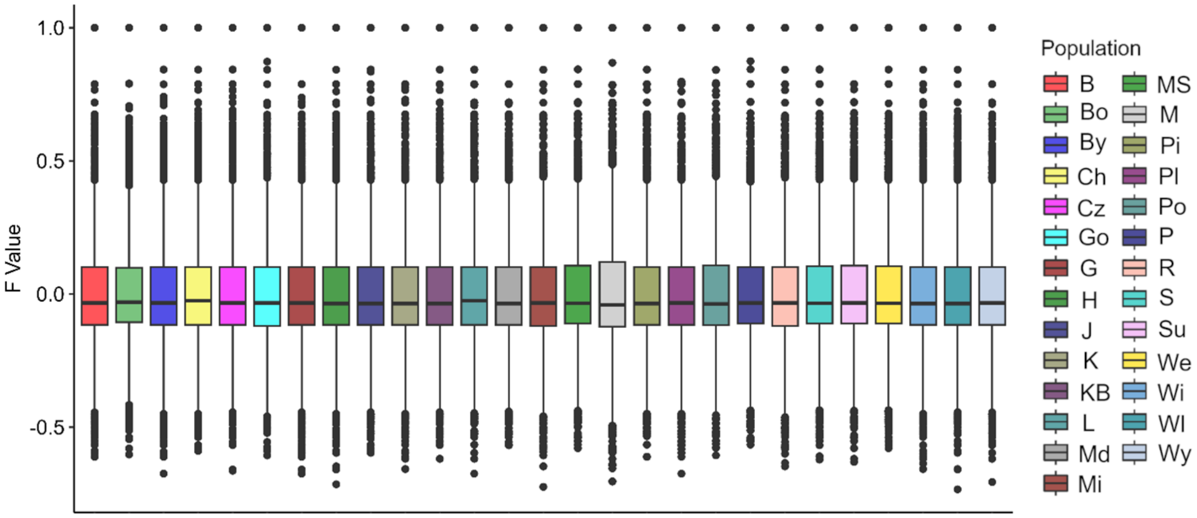
(b)



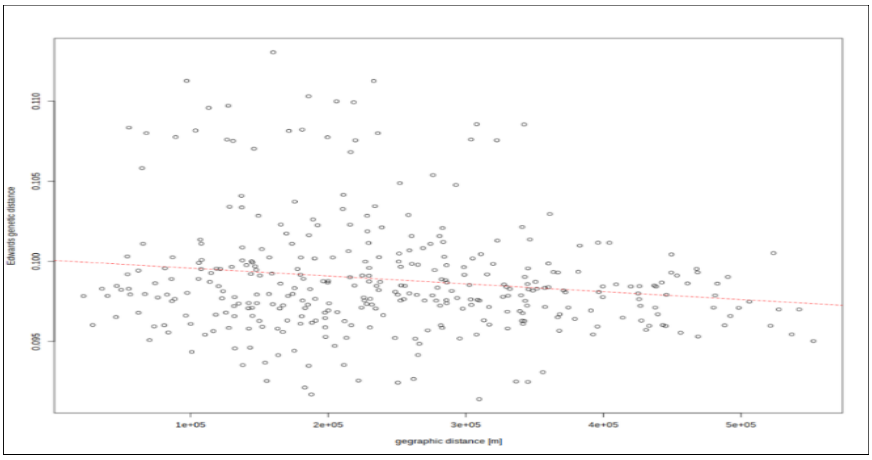
Suppl. Figure S6. Population structure analysis performed using STRUCTURE software



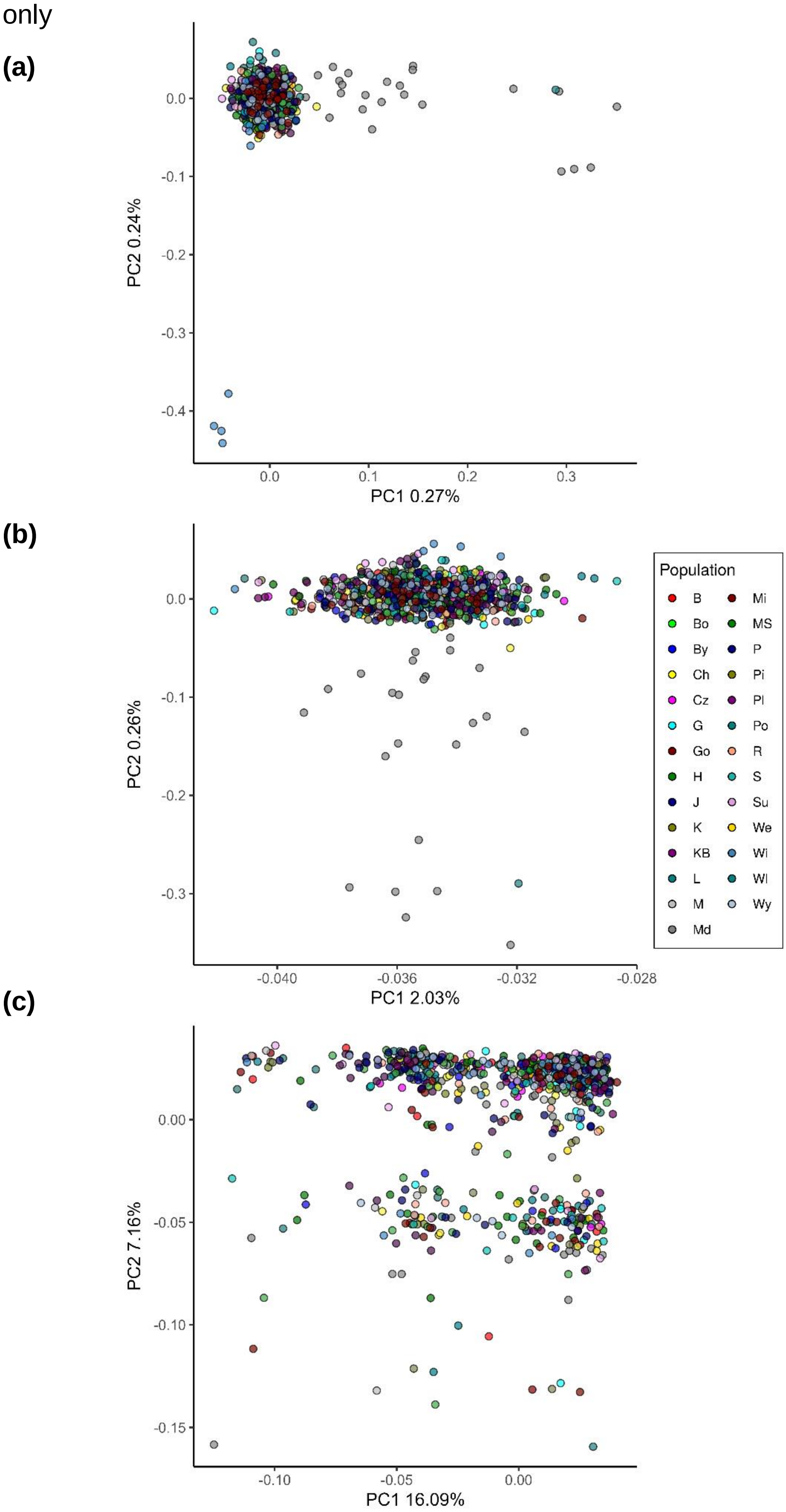
Suppl. Figure S7. Boxplots showing median values of inbreeding across the 27 studied pine populations based on SSR loci



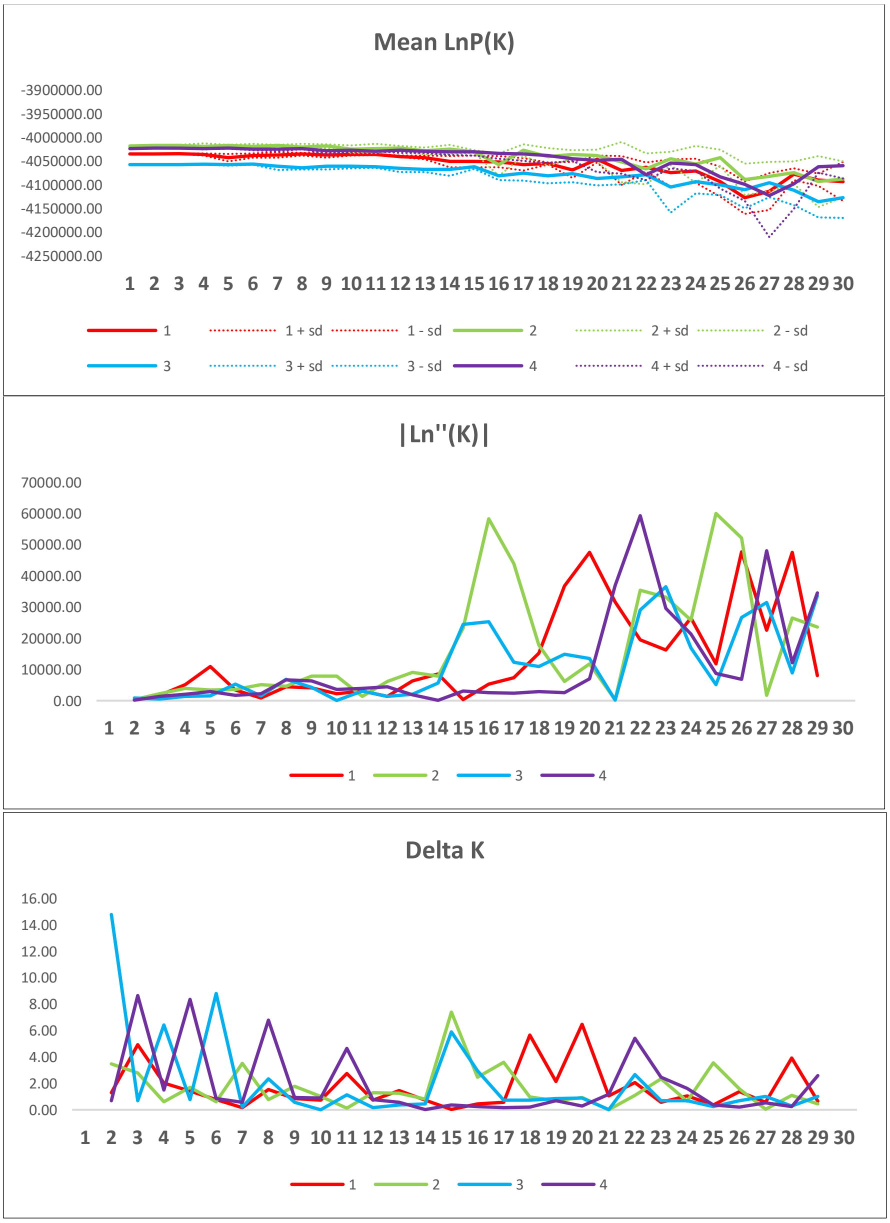
Suppl. Figure S8. Mantel test at SNP markers for the genetic (Edwards distances) and geographic distances between populations ($r^2 = -0.161$, *p*-value = 0.927)



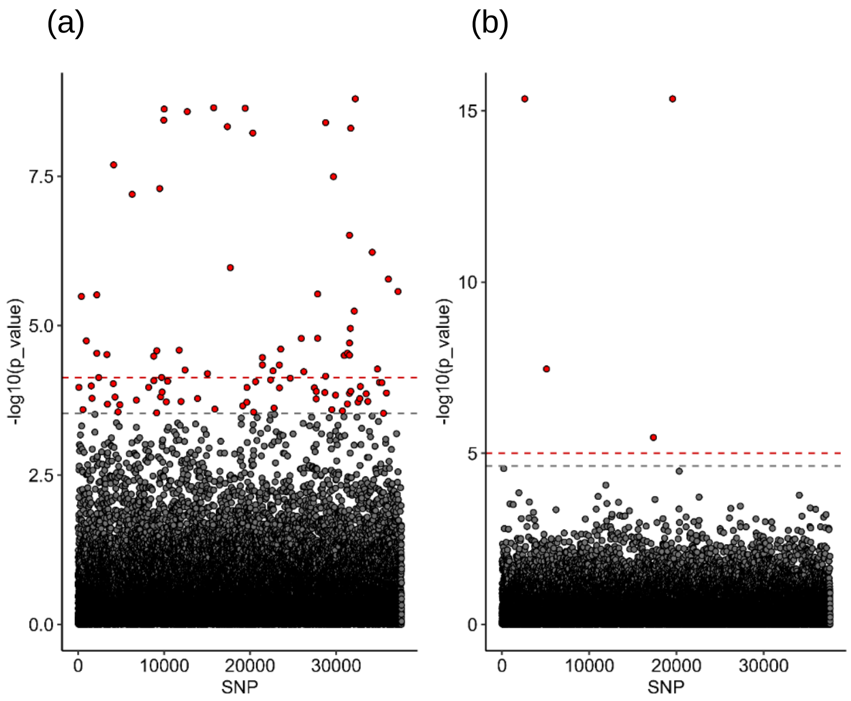
Suppl. Figure S9. Principal Component Analysis (PCA) based on three sets of SNPs: the full set of 37 957 SNPs (a), the set excluding outliers SNPs (b), and the set including only outliers (c)



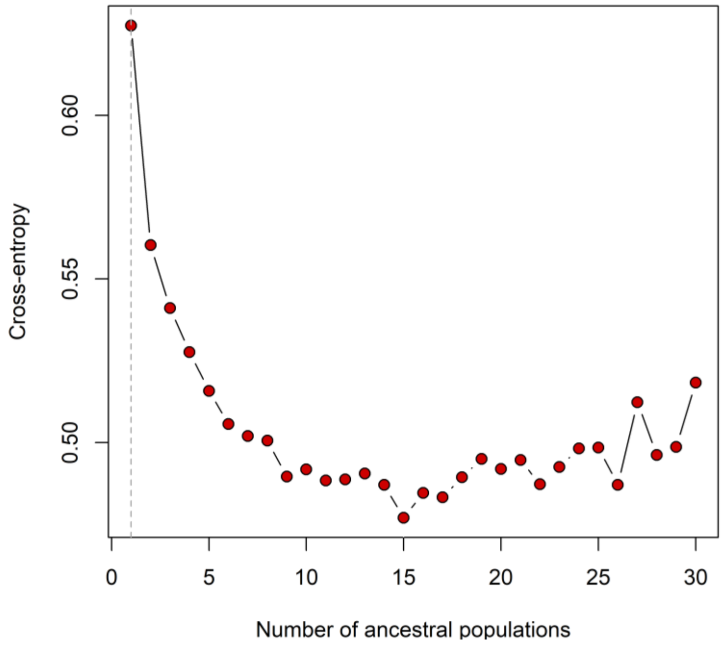
Suppl. Figure S10. Structure analysis (no admixture) results gained by testing 4 subsets of 5,000 random loci from whole SNP set – each represented by different colour. Respective subplots represent main statistics on which inference regarding population genetic structure might be based: lnP(K) mean over 4 replicates; ln’(K); Delta K



Suppl. Figure S11. Manhattan plots showing the outliers SNPs indicated by pcadapt (a) and OutFLANK (b). Red points denote significant outliers SNPs found at both q-value thresholds. The dashed lines indicate the q-value thresholds (grey = 0.1 and red 0.05, respectively)



Suppl. Figure S12. Cross entropy between ten different runs for each K in LEA plotted vs. number of ancestral populations. The optimal number of clusters is detected by the first significant drop of cross entropy at K = 1



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Rozdział 2

Lasek M., Łabiszak B., Wachowiak W. Admixture-Driven Genetic Diversity in Polish Scots Pine Seed Stands: A Blueprint for Climate-Resilient Forestry. **Forest Ecology and Management** – manuskrypt w trakcie procesu redakcyjnego, po pozytywnych recenzjach.

Admixture-driven genetic diversity supports adaptive potential in Scots pine: implications for climate-resilient forest management

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Abstract

Climate change poses a significant threat to the sustainability of European forests, emphasizing the urgent need for informed selection of reproductive material and the conservation of genetic resources in key tree species. Scots pine (*Pinus sylvestris* L.), one of Europe's most widespread and economically important conifers, exhibits broad phenotypic and ecological variation, making it a priority for adaptive forest management. In this study, we investigated the genetic variation of 56 populations comprising Poland's best-performing registered seed stands (PL_RSS), ecologically distinct mountain populations (PLM), and reference populations from Northern, Southern, and Western Europe. A total of 1,269 individuals were genotyped using mitochondrial DNA markers and over 37,000 genome-wide SNPs to infer demographic history, migration, and potential signatures of historical gene flow. Our analyses revealed high genetic diversity and weak population structure within PL_RSS, consistent with a history of admixture among divergent European lineages, as supported by TreeMix, F-statistics, and spatial migration modelling (FEEMS). In contrast, PLM populations were strongly differentiated, carried unique *mt*DNA haplotypes, and exhibited signs of long-term isolation, suggesting a glacial refugial origin. These findings provide the first genomic-scale evidence that historical admixture has enhanced the genetic diversity and potential adaptive capacity of Polish Scots pine seed stands. The admixed and genetically rich PL_RSS populations represent valuable resources for breeding programs, including assisted migration and resilience testing under drought and biotic stress conditions. At the same time, the genetically distinct PLM populations warrant conservation priority due to their unique evolutionary legacy. Together, our results advocate a dual forest management strategy combining the use of high-diversity, well-connected seed sources for adaptive forestry with targeted conservation of isolated mountain populations. This approach is essential for preserving genetic diversity, supporting climate-

resilient forestry, and maintaining the long-term productivity of Scots pine forests in a rapidly changing environment.

1. Introduction

Species range shifts often lead to secondary contact between previously isolated lineages, including both different species and distinct populations within species. While the outcomes of hybridization can vary widely across taxonomic boundaries, contact between conspecific populations typically facilitates gene flow without strong reproductive barriers. Such admixture results in the merging of differentiated gene pools, which can increase genetic diversity, generate novel allelic combinations and genotypes, reduce the impact of deleterious mutations, and enhance adaptive potential in changing environments (Kremer et al., 2012; Yeaman et al., 2016). Despite potential risk such as outbreeding depression or the dilution of locally adapted genomes that may result in temporarily reduced fitness (Lynch, 1991; Frankham et al., 2011; Verhoeven et al., 2011), there are clear examples of intraspecific gene flow promoting population establishments and persistence (Aitken & Whitlock, 2013a). Admixture can manifest in increased growth rates, improved resistance to environmental stress, and enhanced reproductive success. Although the phylogeography of many forest tree species has been well characterized due to advances in molecular methods in recent years, the role of admixture in shaping local ecotypes with valuable phenotypic traits remains insufficiently understood. This knowledge gap is particularly relevant in forestry, where traits such as growth performance, wood quality, and resistance to pests and diseases are key targets for breeding and management. Maintaining adaptive capacity is also becoming increasingly important in the face of climate change and the implementation of assisted gene flow strategies.

Scots pine (*Pinus sylvestris* L.) is a widely distributed foundation species of boreal forest ecosystems of great ecological, social and economic importance (San-Miguel-Ayanz et al., 2016). Its high plasticity and adaptability enable the species to thrive across diverse climates and forest types, from boreal peatlands to dry regions of Caucasus (Dering et al., 2021; Čáp et al., 2023). Historical demographic processes have contributed to the formation of distinct local phenotypes, many of which are highly valued in forestry due to their breeding potential and adaptive traits. Given the extensive range, multiple ecotypes of Scots pine have been identified across its distribution (Giertych, 1991; Białobok et al., 1993). The phenotypic variability and clinal variations in physiological traits, related for instance to photoperiod and temperature (Savolainen et al., 2007), highlights Scots pine's evolutionary capacity for local adaptation, allowing populations to optimize survival and reproduction in specific habitats (Tyrmi et al., 2020).

The high ecotype variability of Scots pine has attracted attention since the early days of forestry science, leading to the first provenance experiments as early as 1824 in France (Von Wangenheim, 1824), followed by a series of international provenance trials (Shutyaev & Giertych, 1997). These long-term studies have consistently demonstrated that Scots pine populations from lowland regions of Europe, including parts of Poland, Germany, Belgium, and the Baltic States, exhibit superior growth performance, broad site tolerance, and high adaptability to diverse conditions. These experiments underscored the importance of local adaptation and genetic background in shaping ecologically and economically significant traits. Additionally, populations from higher elevations and colder climates show increased frost hardiness and drought resistance, making them particularly valuable in the context of environmental change adaptation (Berlin et al., 2016).

In Poland, provenance regions have been delineated to capture the country's most valuable native ecotypes, characterized by traits of particular importance for forest management and breeding (Lasek et al., 2024b). These regions are currently divided into seed zones designed to preserve the genetic integrity and adaptive potential of local populations, thereby facilitating their sustainable use in reforestation and afforestation programs. With increasing pressure to match planting material to future climatic conditions, these zones also serve as a framework for potential assisted migration and breeding strategies. Several studies on IUFRO provenance trials have recommended the inclusion of selected Polish populations, representing local ecotypes as a foundation for future selection and breeding programs (Oleksyn, 1988). Additionally, national studies on provenances from different seed regions have further confirmed the exceptional characteristics of populations, particularly those from Miłomłyn, Rychtal, Supraśl, Bolewice, and Kubryk, which consistently demonstrate favorable performance compared to other sites (Oleksyn, 1988). This strong performance has been linked to the species' postglacial recolonization history and the possibility of gene flow among populations of different origins. However, the hypothesis that admixture has contributed to the genetic composition and performance of Polish breeding populations has not been formally tested, largely due to the previous lack of genomic-scale data capable of resolving fine-scale population structure and historical gene flow.

In this study, we investigated the extent and evolutionary consequence of admixture-driven genetic diversity in Scots pine, focusing on 56 phenotypically and ecologically distinct stands: 27 populations representing Poland's registered seed stands (PL_RSS) across all delineated seed zones, 10 ecologically distinct mountain populations (PLM), and reference populations from the broader European range of the species. Genetic variation was assessed using molecular markers from genomic regions with different modes of inheritance and transmission. Mitochondrial DNA (*mt*DNA) markers were employed to explore maternal lineages and reconstruct past demographic processes. To investigate nuclear variation and assess patterns of gene flow and potential admixture, we conducted high-throughput genotyping of over 37,000 nuclear SNPs. This comprehensive approach enabled us to test the hypothesis that historical admixture among divergent European lineages has increased the genetic diversity and adaptive potential of Polish registered seed stands. It also allowed us to identify the unique evolutionary legacy of southern mountain stands. Finally, we discuss the direct implications of our results for Scots pine forest management, conservation, and breeding efforts in the context of climate-resilient forestry in Europe.

2. Materials and methods

2.1 Plant material and DNA extraction

Scots pine needles were collected from 1,269 individuals representing 56 populations across the European range of the species. Of these, 27 populations originated from Polish registered seed stands (PL_RSS, Figure S1), 10 from protected mountain areas in southern Poland (PLM), and the remaining 19 from reference natural populations in Western (WE), Southern (SE), and Northern Europe (NE) (Table S1). The analysed mountain populations (PLM) were small, typically comprising several dozen to a few hundred trees within limited spatial extents. There is no evidence of their anthropogenic origin, and they are not artificially regenerated. Genomic DNA was extracted from fresh needles following the standard manufacturer's protocol for the Genomic Mini AX Plant kit (A&A Biotechnology, Poland). DNA concentration and quality were evaluated using a BioPhotometer plus (Eppendorf AG, Germany) and adjusted to 40 ng/µl prior to genotyping.

2.2. Genetic data acquisition and basic genetic parameters

Genetic variation was characterized using both mitochondrial and nuclear DNA markers. Mitochondrial DNA (*mt*DNA) is particularly informative in pines due to its maternal inheritance and limited seed-mediated dispersal compared with pollen-mediated markers. A set of 12 *mt*DNA markers (PR: 5, 7, 15, 19, 20, 21, 24, 25, 29, 30, 31, 32 (Donnelly et al., 2017; Łabiszak et al., 2019)) and the *nad1* intron B/C (Soranzo et al., 2000) were genotyped using the SNaPshot method (Szczepański et al., 2023).

Nuclear variation was assessed with the PiSy50k SNP array (Affymetrix, Thermo Fisher Scientific, USA), which includes 49,829 SNPs identified from exome capture, transcriptome sequencing, and targeted resequencing of candidate genes in Scots pine (Kastally et al., 2021). Genotyping was performed using the GeneTitan platform (Affymetrix, Thermo Fisher Scientific, USA) in a 384-well format at Bristol Genomics (UK), following the Axiom Assay protocol. The workflow included DNA amplification, fragmentation, chip hybridization, single-base extension by ligation, and signal amplification. Genotype calls were generated using the Axiom Analysis Suite software under the manufacturer's recommendations. The initial dataset contained 43,565 SNPs. Quality filtering followed standard population genomic practice: individuals with >5% missing data and SNPs that were monomorphic, had a minor allele frequency (MAF) <1%, or significantly deviated from Hardy–Weinberg equilibrium ($p < 0.05$) were removed using PLINK v1.9 (Chang et al., 2015). To minimize linkage disequilibrium (LD), pairwise LD was calculated, and for each pair of SNPs with $r^2 > 0.2$, one SNP was removed using the "--indep-pairwise" and "--r2" options in PLINK. After filtering, 37,597 high-quality SNPs were retained for downstream analyses.

Basic statistics of genetic variation were calculated for *mt*DNA and nuclear datasets. For *mt*DNA, the number of haplotypes (H), effective number of haplotypes (N_e), haplotypic richness (R_h), and haplotype diversity (H_d) were computed using HaplotypeAnalysis v1.05 (Eliades & Eliades, 2009). Nuclear SNP diversity was summarized as observed (H_o) and expected heterozygosity (H_e) and the inbreeding coefficient (F_{IS}) using the adegenet and hierfstat packages in R v4.2 (Goudet, 2005; Jombart, 2008; Team, 2025).

2.3. Phylogeography and population structure

Phylogenetic relationships among *mt*DNA haplotypes were inferred in POPART v1.7 (Leigh & Bryant, 2015). The resulting haplotype network was used to visualize maternal lineages and infer phylogeographic relationships among populations. The spatial distribution of *mt*DNA haplotypes (mitotypes) was visualized using QGIS v3.36, while multilocus genotypes were analysed to assess population structure. Relationships among populations were quantified using Nei's genetic distance, and a hierarchical clustering tree was constructed with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) implemented in R v4.2. The resulting tree was visualized with FigTree v1.4.4 (Rambaut, 2018).

Pairwise F_{ST} values were calculated between geographic regions and displayed as a heatmap using the corrplot package (Wei et al., 2017; Team, 2022). Population structure was further evaluated with Principal Component Analysis (PCA) and likelihood-based clustering implemented through sparse non-negative matrix factorization (SNMF) in the LEA package (Frichot & François, 2015). The optimal number of genetic clusters (K) was determined by minimizing the cross-entropy criterion across K = 1–10, and the results were visualized using the pophelper package (Francis, 2017).

2.4. Admixture and migration inference based on *n*SNPs

Spatial variation in gene flow was modeled using Fast Estimation of Effective Migration Surfaces (FEEMS) (Marcus et al., 2021). Genotype data were filtered to remove monomorphic and missing loci, then standardized using *z*-score scaling. Sampling locations were projected onto an Equidistant Conic projection centered on Central Europe (19.39°E, 52.73°N). A spatial graph was constructed by connecting nodes within a buffered region around sampling sites. To delinate the study area, an alpha-hull polygon was generated and expanded with a small buffer to minimize edge effects and spatial bias. Models were fit across a range of regularization parameters (λ), and the optimal value was selected by minimizing cross-validation error. The final migration surface was visualized using a log-transformed colour scale representing relative effective migration rates ($log_{10}w$).

Historical relationships and long distance gene flow among Scots pine populations were analysed with TreeMix v1.13 (Pickrell & Pritchard, 2012). TreeMix models allele frequency covariance as a function of shared genetic drift, with optional migration edges representing admixture events. For these analyses, populations were aggregated into five regional clusters (PL_RSS, PLM, WE, SE, NE). TreeMix was run without a defined outgroup (–root noRoot) and with up to 10 migration edges (m = 0–10), using five replicates per m and a block-jackknife over 100 SNPs (k = 100) to account for LD. Additional runs using k = 10 and k = 1000 SNPs were performed to verify the stability of likelihood surfaces and migration edge inferences. The automated bootstrap pipeline and TreeMix extension scripts provided by Dahms et al. (2025) were used for consensus tree construction and model validation.

The optimal number of migration edges was determined using OptM package (Fitak, 2021), which implements AIC-based model selection to compare alternative TreeMix fits. The final maximum-likelihood tree was chosen using the maxLL() function, which selects the topology with the highest likelihood across replicates. Pairwise drift and residual matrices were extracted from the TreeMix outputs. Pairwise drift estimates reflect the amount of shared genetic drift, whereas residuals identify deviations from the tree model, suggesting additional structure or gene flow.

To assess shared ancestry and admixture, we calculated f3 and f4 statistics using the admixtools R package (Maier & Patterson, 2024). These complementary statistics allowed us to assess both shared ancestry and deviations from a simple tree-like model of population splits. The f3-statistics were calculated in the form f3 (A; B, C) for all relevant triplet combinations among predefined population groups. Block-jackknife resampling was used to estimate standard errors, with genomic blocks defined by a fixed number of SNPs (blgsize = -5000) to accommodate uneven marker distribution. Significant negative f3 values would indicate admixture in population A from sources related to B and C, whereas significantly positive values reflect shared drift among the tested populations. Complementary, f4-statistics were computed in the form f4 (A, B; C, D) to formally test whether two pairs of populations exhibit symmetric allele sharing, which would be expected under a simple bifurcating tree. Significant deviations from zero indicate either gene flow or an incorrect tree topology. These tests are particularly useful for detecting subtle or ancient gene flow events that may not be evident from f3-statistics alone. All data handling was performed in dartR (Gruber et al., 2018) and visualization in ggplot2 (Wickham, 2016).

3. Results

3.1 Genetic diversity and differentiation

Out of 13 *mt*DNA markers analysed, 11 were polymorphic, providing 24 distinct haplotypes. However, only the 13 haplotypes that occurred in more than three individuals were used to recreate the haplotype network and illustrate their geographic distribution (Figure 1). Two major haplogroups were identified, with the most frequent haplotypes – H1 and H2. Both of them were present in approximately 70% of all sampled trees (525 and 395 individuals, respectively). Similarly, UPGMA analysis of haplotype relationships revealed two primary haplogroups (Figure S2). The next most common haplotypes, H3 (139 individuals) and H10 (57) were closely related with H1, as well as several other low-frequency haplotypes. Most of the Polish registered seed stands populations exhibited high haplotype richness (Table 1, Figure S3). Some populations were fixed for a single haplotype (e.g. PNP, FIN12). Nuclear SNP markers showed moderate but consistent levels of genetic diversity across populations, with no signatures of deviation from HWE and near-zero inbreeding coefficient (F_{IS}) in most cases (Table 1, S2 and Figure S4). Overall, both mitochondrial and nuclear datasets indicate high within-population diversity and low differentiation among PL_RSS populations, consistent with widespread gene flow and historical admixture.

3.2 Population structure and phylogeographic patterns

Although mitochondrial haplotypes H1 and H2 are not closely related, both were present in all Polish registered seed stands (PL_RSS) populations (Figure 1). Haplotype H2 is predominant in the Fennoscandian region, where most populations are characterized by either complete fixation of H2 or a minor admixture of other mitotypes. Several unique haplotypes were found in PLM, indicating historical isolation and limited maternal gene flow. The Pieniny National population was fixed for H1, whereas the Tatra population from Wielkie Koryciska (WK) harbored a haplotype otherwise found only in Swiss and French stands. The highest genetic divergence measured by pairwise F_{ST} at SNP loci were observed between groups of Northern Europe (NE) and Western Europe (WE) as well as between Polish mountain (PLM) and Western Europe (F_{ST}=0.01 in both cases). In contrast, the lowest differentiation was observed between Polish registered seed stands and Southern Europe (SE) (F_{ST}=0.001, Figure S5). Principal Component Analysis (PCA) showed that individuals from PLM populations, particularly WK and DBS, were clear outliers, whereas the remaining populations formed a tight genetic cluster with minimal dispersion (Figure 2, Figure S6). To minimize the influence of strong genetic divergence in PLM, all Polish mountain populations (PLM) were excluded and the PCA was repeated. After removing PLM, clearer differentiation emerged among the Northern European, Western European and Polish registered seed stands populations, with the later overlapping to some extent with Southern European samples (Figure 2). A similar clustering pattern was observed after subsampling to control for the overrepresentation of the PL_RSS group. The analyses indicated that the underlying population structure was not an artifact of unequal sample size (Figure S7).

Population clustering results were consistent with PCA patterns. The optimal number of ancestral populations was determined to be K=7, based on cross-entropy criterion (Figure 3, S8). Notably, most PLM populations formed clearly differentiated genetic clusters, while some of these mountain populations exhibited varying degrees of admixture. This analysis markedly delineates the PLM populations as distinct genetic groups, revealed subtle differentiation of the Finnish populations, and confirmed overall homogenity of genetic structure in the remaining populations.

3.3. Gene flow and admixture inference

We used FEEMS to explore how spatial structure and geographic features influence gene flow across the Scots pine range. The final surface was generated using a regularization parameter $\lambda = 5.45$, selected through cross-validation to balance model fit and smoothness, ensuring biologically meaningful patterns while minimizing overfitting (Figure S9). The inferred migration surface (Figure 4) highlights a complex landscape of connectivity, with marked spatial heterogeneity in effective migration rates. Migration was highest across Central and Eastern Europe, particularly through Poland and the Baltic corridor, as indicated by blue-hued edges ($\log_{10}(w) > 1$). These areas correspond with dense sampling and likely reflect well-connected population structure.

In contrast, reduced migration (orange edges, $\log_{10}(w) < 0$) is evident in regions to the west and south, especially around the Polish Mountains populations (PLM) and extending into Southern Europe (SE). This spatial barrier aligns with the extended branch length, and with the elevated pairwise drift estimated for PLM, as well as with the consistently high f3 values obtained when PLM serves as the outgroup. FEEMS also identified low connectivity among northern Scandinavian populations, forming a potential barrier to gene flow in the north.

To further explore the population history, we constructed a consensus tree of population relationships using TreeMix, allowing for 0 to 10 migration events (m). However, adding migration edges beyond 3 did not improve model and was omitted in final runs. Across five replicate runs per each migration level ($m = 1–3$), TreeMix returned consistent tree topologies and log-likelihoods, indicating a strong signal and model stability. Likelihoods increased from $m = 1$ to $m = 2$, however the gain from $m = 2$ to $m = 3$ was marginal (+0.11 log-units). This indicates that further increases in 'm' are unlikely to yield substantially different topologies or better fit. Model selection with the OptM package confirmed that the most informative model included one or two migration edges. The Piecewise Linear model had the lowest AIC (–7.80), identifying an inflection point at $m \approx 1.3$, supporting $m = 2$ as the most parsimonious model (Figure S10, Table S3).

The inferred TreeMix graph with two migration edges revealed key features of population history (Figure 5). The first migration event originated from the ancestral lineage of NE and WE and was directed toward PL_RSS. The second migration event originated from WE and was directed toward the internal branch ancestral to PL_RSS and SE. Drift parameter matrices (Figure 5) indicated the highest drift accumulation in PLM and SE, while PL_RSS and NE exhibited lower drift values, suggesting more recent gene flow or larger effective population sizes. Residual plots (Figure S11) showed generally low model misfit, with localized positive residuals between PLM, PL_RSS, and SE, corresponding to the inferred migration edges.

We used f3-statistics to evaluate patterns of shared genetic drift and potential admixture among *P. sylvestris* populations across Europe. All tested combinations yielded significantly positive f3-values (Figure 5, Supplementary Table S4), suggesting no evidence of recent large-scale admixture between major population groups. Among all regions used as outgroups, PLM consistently produced the highest f_3-values across triplet comparisons, indicating that this region is the most genetically divergent and shares the least ancestry with the others. Additionally, the lowest level of shared drift with PLM as outgroup was recorded in combination with NE and SE, followed by the PL_RSS and NE with significantly higher values in all possible triplets with WE. In contrast, Polish registered seed stand populations showed the lowest f3-values across all comparisons and weak structuring, suggesting lower divergence, or complex origins relative to other groups. Strikingly, the lowest value of f3 statistic was scored for the same combination of regional groups as with PLM - SE and NE. Triplets involving NE and SE populations yielded moderately high f3 values, indicating substantial shared drift and possibly a common postglacial history.

The fifteen f4-statistics revealed a clear hierarchy of allele sharing inconsistent with a single bifurcating population tree (Table S5). Twelve of fifteen comparisons were significant ($|Z| > 3$), indicating widespread asymmetry in allele sharing among the five regional groups. The strongest signals of asymmetry involved PL_RSS. For instance, f4 (SE, WE; PL_RSS, NE) = 2.97×10^{-4} ($Z = 15.2$) indicates excess allele sharing between PL_RSS and SE relative to NE. The reciprocal comparison, f4(SE, NE; WE, PL_RSS) = -2.19×10^{-4} ($Z = -8.8$), reveals a greater of allele sharing between PL_RSS and WE. Together, these results demonstrate that Polish registered seed stands (PL_RSS) represent a genetic mosaic formed through admixture of distinct European lineages, while mountain populations (PLM) maintain unique, less admixed genetic signatures likely shaped by historical isolation.

4. Discussion

4.1. Admixture and historical relationships among Polish registered seed stands of Scots pine

Despite previously described phenotypic distinctions, our genomic analyses revealed high genetic similarity among the studied Polish registered seed stands (PL_RSS), suggesting a shared postglacial recolonization history and widespread historical connectivity across the Polish lowlands. Mitochondrial DNA analyses revealed elevated haplotype richness relative to other European regions. The observed *mt*DNA diversity indicates multiple maternal lineages co-occurring within Poland, consistent with recolonization from several glacial refugia. The higher haplotypic diversity in lowland seed stands than in mountain populations suggests that PL_RSS have integrated genetic material from multiple European sources, enhancing their evolutionary potential.

This pattern of haplotype co-distribution is supported by dense nuclear SNP data. High within-population diversity and limited interpopulation differentiation suggest widespread historical gene flow and shared post-glacial recolonization history (Gömöry et al., 2023; Wachowiak et al., 2023; Bruxaux et al., 2024). The results suggest that Polish registered seed stands may have formed through admixture among distinct genetic lineages, followed by seed exchange among regions. Although an anthropogenic origin of some populations cannot be fully excluded, given documented seed transfers across Europe during the 19th and early 20th centuries (Myking et al., 2016; Jansen et al., 2017; Jansen et al., 2019), the natural origin of most PL_RSS stands is strongly supported by their age (~180 years), uniform genetic structure, and consistent dominance of two major haplotypes across all populations. Moreover, historical partitioning of Poland under Prussian, Russian, and Austrian governance resulted in distinct forestry administrations, reducing the likelihood of large-scale artificial mixing. These factors collectively suggest that the PL_RSS are most probably of natural origin.

Our integrative analysis combining TreeMix, f-statistics, and spatial migration modelling reveals complex patterns of historical and potentially ongoing admixture among *P. sylvestris* populations in central and marginal regions of Europe. These patterns highlight contrasting demographic trajectories among mountain refugia, recolonization corridors, and admixed Polish seed stands. Overall, the PL_RSS populations displayed signs of complex ancestry and weak genetic structure. TreeMix inferred two migration edges targeting the lineage leading to PL_RSS: one from the ancestral NE–WE branch and another directly from WE. F_4-statistics confirmed asymmetric allele sharing with both SE and NE, consistent with subtle but widespread admixture events. Notably, PL_RSS had the lowest f3-values among all tested combinations, consistent with a history of mixed ancestry from divergent lineages. This genomic pattern resembles a hybrid zone dynamic, where previously isolated postglacial lineages interbred during secondary contact, generating novel alleles combinations of potential adaptive significance (Ortego et al., 2012; Abbott et al., 2013). Such admixture zones have been recognized as evolutionary hotspots that enhance adaptive potential through increased heterozygosity and transgressive segregation of traits (Aitken et al., 2008; Hamilton & Miller, 2016b)

One plausible interpretation is that PL_RSS genomic composition reflects contributions from southern refugial sources (via SE) and a potentially unsampled eastern lineage contributing to the NE cluster - a 'ghost' population scenario. Such ancestry could mimic admixture-like signals between NE and SE in triplet tests involving PL_RSS. FEEMS results further support this interpretation, revealing high effective migration across Poland and the Baltic corridor, with a distinct path of elevated connectivity linking WE to PL_RSS. This spatial pattern parallels the admixture pathways suggested by TreeMix and f_4-statistics. Interestingly, the inferred gene flow corridors coincide with lowland plains and river valleys (e.g. Vistula and Oder basins), which historically facilitated species migration and dispersal (Svenning et al., 2008). Such landscapes may have acted as suitable distribution paths during both natural recolonization and incidental anthropogenic seed movement. The detection of multiple migration edges and significant f4 asymmetries (e.g., f4(SE, WE; PL_RSS, NE)) imply that Polish stands represent a genomic mosaic that appears to be shaped by multiple processes, including admixture from geographically and genetically distinct sources and potential landscape-mediated introgression along natural corridors.

From a forestry perspective, this mixed ancestry likely contributes to the superior performance of PL_RSS populations in provenance trials (Giertych, 1979; Giertych, 1997; Oleksyn et al., 1998). Genetic admixture has been shown to enhance traits such as growth, stress tolerance, and reproductive success (Verhoeven et al., 2011; Hamilton & Miller, 2016a). Additionally, populations that integrate alleles from multiple lineages often display broader climatic envelopes and increased resilience to stress (Aitken & Whitlock, 2013b; Hamilton & Miller, 2016b). As a result, these stands constitute versatile seed sources with the potential to buffer productivity losses expected under near-term climate warming. This supports their use as broadly adapted sources in assisted migration and reforestation efforts aimed at enhancing climate resilience. Our FEEMS analysis unveils a network of elevated gene flow corridors in Central and Eastern Europe, contrasting with reduced connectivity in mountain and southern regions. That patterns align with landscape-level assessments of *P. sylvestris* connectivity (Wachowiak et al., 2022).

4.2. Distinct Genetic Identity of Mountain Populations

In contrast, the distinct *mt*DNA haplotypes and higher F_{ST} values in mountain populations (PLM) point to long-term isolation and limited maternal gene flow. These populations likely represent remnants of ancient refugial lineages that persisted under local climatic stability. In the PCA and population structure analyses based on SNP data, PLM populations are confirmed to form genetically distinct groups, and some of them exhibited varying degrees of admixture. Notably, the remaining studied populations across Europe appear much more tightly clustered, with only slight differentiation observed in the Fennoscandian region. PLM emerged as the most genetically divergent group, showing strong drift signals in TreeMix and consistently elevated f_3-statistics when used as an outgroup. This indicates a long-term isolation or distinct glacial origin, likely reinforced by ecological and topographic barriers, as reflected in the reduced effective migration surrounding the PLM stands in the FEEMS surface.

These genetic patterns align with previous studies that identify European mountain ranges, including the Carpathians and Alps, as glacial refugia for cold-adapted species during the Last Glacial Maximum (LGM), with post-glacial recolonization expanding from these refugial zones (Cheddadi et al., 2006; Tzedakis et al., 2013). Such areas are often characterized by the retention of ancient lineages and higher genetic differentiation, due to restricted gene flow and local adaptation. In Scots pine, previous mitochondrial DNA evidence has also indicated mixed or cryptic refugia in Central and Eastern Europe (Pyhäjärvi et al., 2007; Pyhäjärvi et al., 2008; Dering et al., 2017; Wachowiak et al., 2023; Żukowska et al., 2023), supporting the hypothesis that the PLM stands represent genetically unique, historically persistent lineages. Topographic complexity, steep environmental gradients, and edaphic heterogeneity in mountainous landscapes may further promote population subdivision and maintain divergent genotypes by limiting pollen and seed dispersal (Hampe & Petit, 2005; Holderegger & Wagner, 2006). The low connectivity inferred by FEEMS around PLM populations likely reflects both historical isolation and present-day barriers to gene flow, such as elevation, aspect, and climatic contrasts between mountain valleys and ridgelines.

From a conservation genetics perspective, mountain populations serve as a unique reservoirs of rare alleles and locally adapted gene complexes, which are critical for maintaining long-term evolutionary potential under climate change (Aitken et al., 2008; Frankham et al., 2011). Their persistence ensures that marginal environments remain occupied by lineages capable of surviving harsh and fluctuating conditions. These findings position PLM as a likely refugial remnant lineage, consistent with border pattern of mountainous regions acting as long-term genetic diversity reservoirs through glacial persistence and limited connectivity. We therefore recommend prioritizing these populations for in situ conservation and considering them as distinct Conservation Units (CUs) under national forest genetic resource management strategies, to ensure the maintenance of gene complexes critical for stand stability in high-elevation environments. Additionally, reciprocal transplant and common garden trials should be implemented to test the degree of local adaptation and phenotypic plasticity in PLM populations. Such experiments could identify genotypes with enhanced stress resilience, informing future breeding programs aimed at maintaining Scots pine adaptability in changing climates.

4.3. Implications for Conservation, Forestry and Breeding

Effective management of Scots pine, one of the most economically important timber species in Europe, is increasingly challenging under rapid environmental change. Ongoing climatic shifts are already affecting forest productivity through reduced fitness, drought stress, pathogen outbreaks, and increased mortality in parts of the its’ range. Integrating genomic information with climate projections and adaptive trials is therefore essential for identifying resilient genetic resources and guiding future afforestation and breeding strategies (Aitken & Whitlock, 2013a; Alfaro et al., 2014).

Maintaining high genetic diversity is central to adaptive capacity and long-term population resilience. In this context, the high genetic diversity and near-zero nuclear inbreeding observed in Polish registered seed stands (PL_RSS) indicate substantial evolutionary potential. These populations represent an important genetic resource for forestry, breeding, and restoration, particularly under projected warming and drought scenarios (Matyas, 1996; Pedlar et al., 2011). However, the current subdivision of Polish lowland populations into numerous regions of provenance does not reflect their largely homogeneous genetic background and broadly similar climatic conditions, suggesting that existing seed zone delineations require revision (Lasek et al., 2024a). In contrast, the mountain stands (PLM), exhibit strong genetic differentiation and likely represent long-term, locally adapted lineages. Their restricted distribution, small population sizes, and unique genetic composition highlight their importance for conservation. From a management perspective, these populations may harbor adaptive traits relevant for stress tolerance, emphasizing the need for targeted in situ protection and genetic monitoring.

Together, our results support a dual management strategy: (i) the use of genetically diverse and well-connected lowland populations (PL_RSS) as seed sources for reforestation, breeding, and assisted migration, accompanied by streamlined and genetically informed seed zoning; and (ii) the prioritization of conservation efforts for isolated mountain populations (PLM) to safeguard their unique evolutionary legacy. Incorporating this framework into national and EU-level policies on forest reproductive material (Commission, 2023) and the FAO's updated global guidelines for forest genetic resource management (FAO, 2025) would strengthen adaptive forest management and long-term resilience across Central Europe.

5. Conclusions

Our study shows that Polish registered seed stands (PL_RSS) of Scots pine represent genetically diverse populations shaped by admixture among divergent European lineages, resulting in weak population structure and high adaptive potential. In contrast, mountain populations (PLM) are genetically distinct, weakly admixed, and likely reflect long-term isolation and local adaptation. These contrasting genomic patterns support a dual management approach that combines the use of diverse lowland seed sources for adaptive forestry with targeted conservation of isolated mountain lineages. Revising current seed zone delineations based on genomic evidence will be critical for aligning forest management practices with evolutionary processes and improving the resilience of Scots pine forests under ongoing climate change.

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Conflict of Interest

The authors declare no conflict of interest.

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Tabel 1. Basic statistics at mitochondrial (*mt*DNA) and nuclear (*n*SNP) markers for the analysed populations are divided into distinct geographical locations. Abbreviations: N - number of samples; H - number of haplotypes; N_e - effective number of haplotypes; R_h - haplotypic richness; H_d - haplotype diversity; H_o - observed heterozygosity; H_e - expected heterozygosity; F_{IS} - averaged inbreeding coefficient.

Population region		***mt*DNA**				***n*SNP**		
(Acronym)	N	H	N_e	R_h	H_d	H_o	H_e	F_{IS}
Polish registered seed stands (PL_RSS)	840	5.741	2.935	1.793	0.662	0.294	0.294	0.001
Western Europe (WE)	37	2.000	1.480	0.664	0.319	0.287	0.288	-0.003
Southern Europe (SE)	39	2.250	1.869	1.080	0.476	0.292	0.289	-0.020
Northern Europe (NE)	132	2.500	1.747	0.903	0.343	0.298	0.296	-0.009
Poland mountains (PLM)	221	2.900	1.821	0.860	0.328	0.289	0.287	-0.006
Total / Average	1269	3.882	2.273	1.287	0.499	0.292	0.292	-0.005

Figure 1. (a) Network of major mitochondrial DNA (*mt*DNA) haplotypes identified across studied populations. Circle sizes are proportional to haplotype frequencies; colours correspond to haplotypes shown in maps (b–d). Geographic distribution of *mt*DNA haplotypes across European populations (b), Polish registered seed stand populations (PL_RSS) (c), and in the Polish mountain regions (PLM) (d). Boundaries of the analysed Polish Scots pine seed provenance regions are marked with black line (c) and are presented also in Supplementary Figure S1 based on the data from the Polish Forest Seed Office https:// www. bnl. gov. pl/.

a)

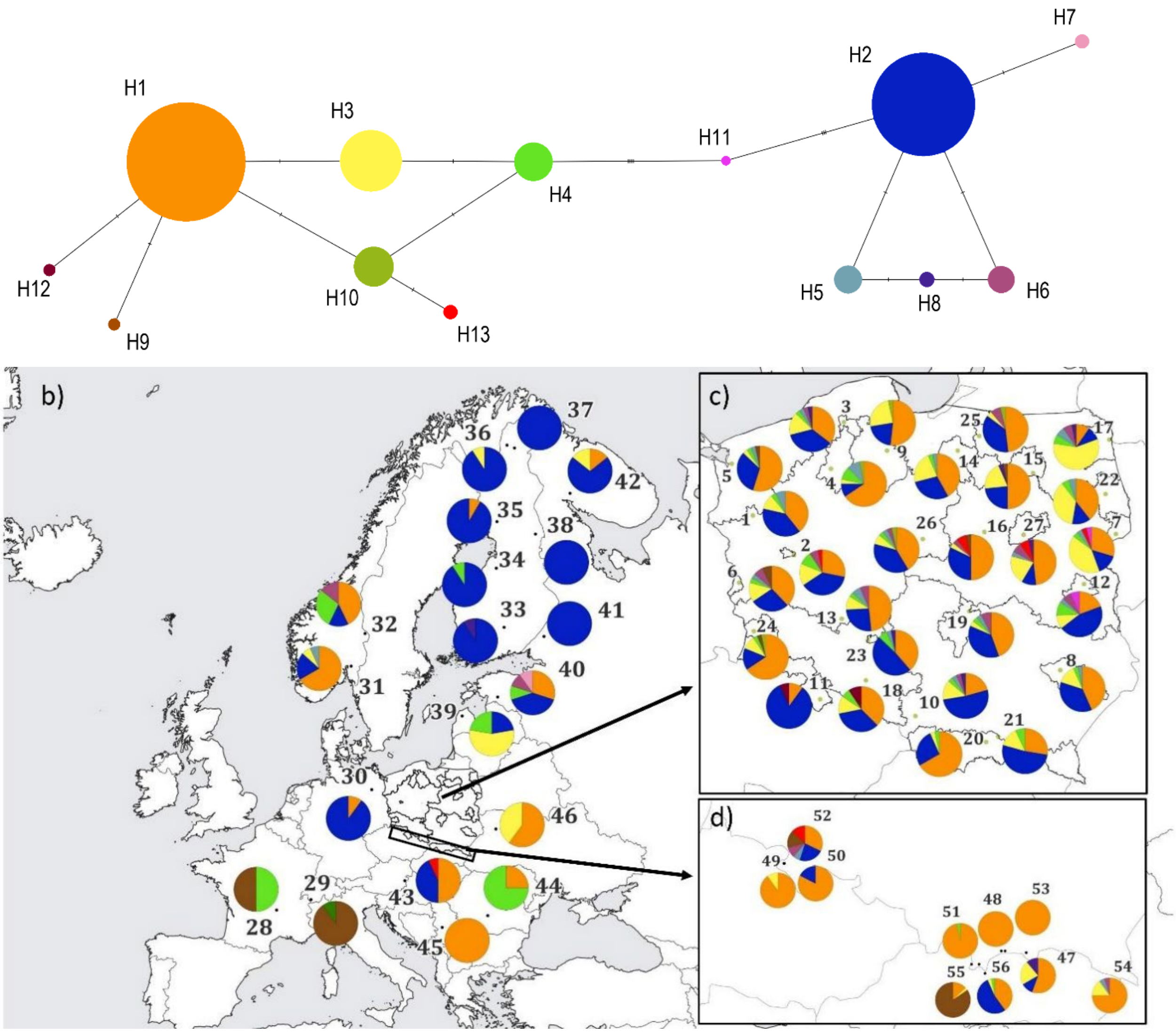


Figure 2. Principal Components Analysis (PCA) based on SNP marker data, with populations grouped according to geographical regions, including all populations (a) and after exclusion of all Polish mountain populations (PLM) (b) (see also Figure S6 for more details). Different colours indicate distinct regions: NE – Northern European stands (blue), PL_RSS – Polish registered seed stands (green), PLM – Polish mountain stands (red), SE – Southern European stands (yellow), and WE – Western European stands (purple).

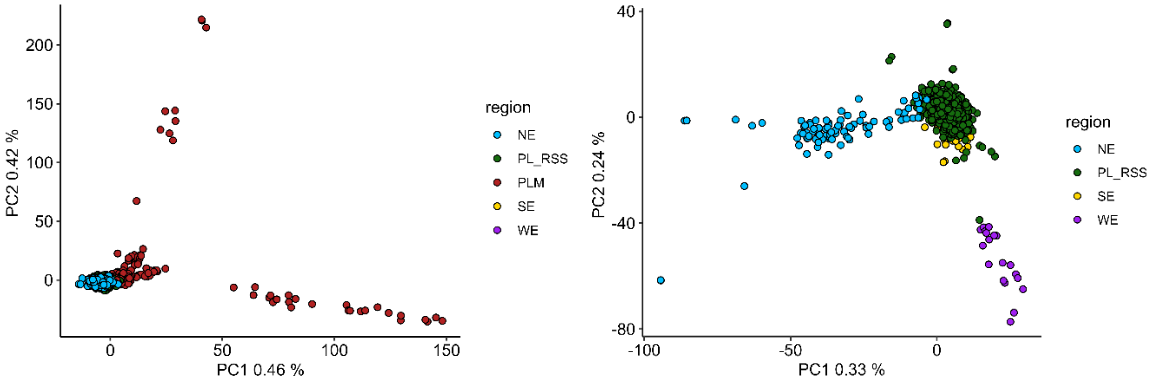


Figure 3. Population genetic structure of all analysed populations based on SNPs markers, inferred using the LEA at the optimal number of clusters (K = 7; see Figure S8). Population codes correspond to sampling locations listed in Table S2.

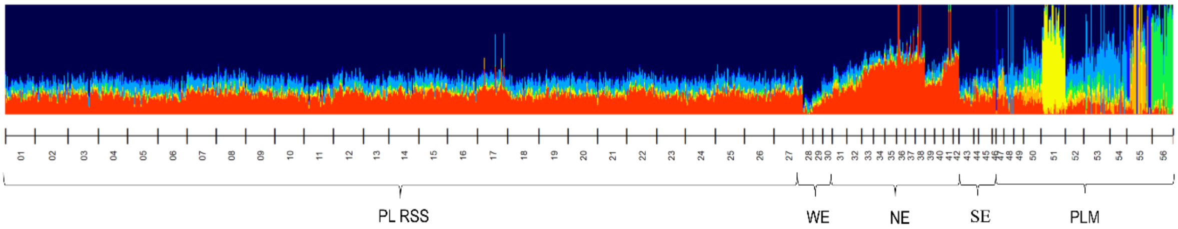


Figure 4. Effective migration surface inferred using FEEMS. Spatial estimates of effective migration rates across the range of sampled *Pinus sylvestris* populations. Values are shown as log10 transformed effective migration rates (*w*) along edges of a spatial graph overlaid on an equidistant conic projection. Sampling locations are represented by the black nodes. The colour gradient reflects relative rates of migration, with orange tones indicating lower effective migration and blue tones indicating higher rates. Model fitted using λ = 5.45, selected via cross-validation (see Figure S9).

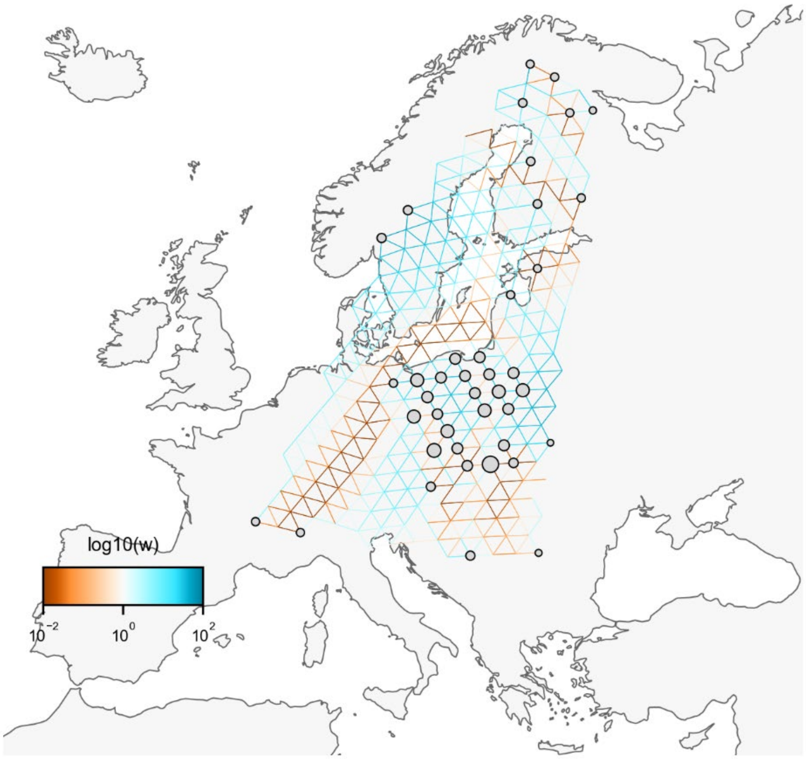
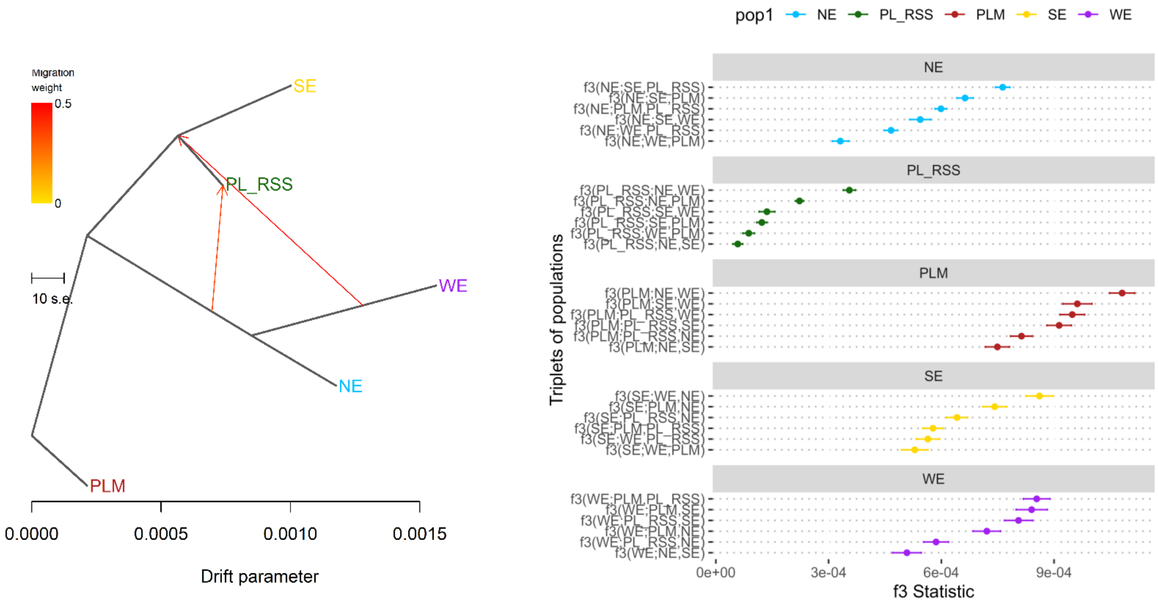


Figure 5. Shared drift and admixture patterns among five regional clusters of Scots pine. Left panel: TreeMix maximum-likelihood graph with two migration edges (m = 2), shown with drift-scaled branch lengths and coloured migration arrows indicating weight. Tree constructed using allele count data grouped into five regions. Right panel: Results of *f*$_3$-statistics test calculated in the form *f*$_3$(A; B, C) for all relevant triplet combinations among five predefined population clusters (NE, PL_RSS, PLM, SE, WE), using block jackknife resampling. Values are plotted with 2× standard error bars; all triplets yielded significantly positive f$_3$ values.



Supplementary Materials

Table S1. Geographic information for the studied Scots pine (*Pinus sylvestris*) populations. Shown are the population names, acronyms, number of individuals, geographic regions (PL_RSS – Polish Reference Seed Stands; WE – Western Europe; NE – Northern Europe; SE – Southern Europe; PLM – Polish Mountain populations), latitude, longitude, and elevation (in meters above sea level) of each sampling location.

Population	Acronym	Indviduals	Region	Latitude	Longitude	Elevation
Barlinek	B	30	PL_RSS	52.93	15.18	101
Bolewice	Bo	36	PL_RSS	52.38	16.13	103
Bytów	By	31	PL_RSS	54.22	17.29	121
Czarne_Czl.	Cz	30	PL_RSS	53.58	16.99	146
Goleniów	Go	31	PL_RSS	53.65	14.70	18
Gubin	G	32	PL_RSS	51.98	14.86	71
Hajnówka	H	30	PL_RSS	52.62	23.60	180
Janów_Lub.	J	30	PL_RSS	50.71	22.31	224
Kaliska	K	30	PL_RSS	53.83	18.29	125
Kobiór	Kb	30	PL_RSS	50.03	18.95	273
Międzylesie	Md	33	PL_RSS	50.29	16.74	547
Międzyrzec	MS	31	PL_RSS	51.97	22.87	164
Milicz	M	32	PL_RSS	51.46	17.24	198
Miłomłyn	Mi	31	PL_RSS	53.83	19.93	147
Pisz	Pi	30	PL_RSS	53.52	21.68	136
Płońsk	Pl	30	PL_RSS	52.7	20.55	131
Pomorze	Po	32	PL_RSS	53.98	23.45	150
Prószków	P	32	PL_RSS	50.56	17.80	208
Spała	S	31	PL_RSS	51.57	20.20	201
Chełmiec	Ch	32	PL_RSS	49.64	20.59	532
Lipnica	L	30	PL_RSS	49.72	20.87	503
Supraśl	Su	31	PL_RSS	53.23	23.36	176
Rychtal	R	34	PL_RSS	51.19	17.96	217
Węgliniec	We	32	PL_RSS	51.29	15.20	201
Wichrowo	Wi	30	PL_RSS	54.03	20.43	110
Włocławek	Wl	30	PL_RSS	52.6	19.12	69
Wyszków	Wy	30	PL_RSS	52.66	21.47	129
France Arlanc Mayers	FRA	12	WE	45.4	3.69	838
Switzerland Follatères	CHE	15	WE	46.12	7.08	660
Germany Godendorf	GER	10	WE	53.15	13.08	84
Norway Østmarka	NO2	14	NE	59.87	11.05	330
Norway Smoldalen	NO3	14	NE	61.45	12.42	660
Finland Joutsa	FIN1	12	NE	61.74	26.14	105
Finland Temmes and Tyrnävä	FIN2	12	NE	64.39	25.37	40
Finland Rovaniemi	FIN3	12	NE	66.31	25.44	76
Finland Kielajoki	FIN4	12	NE	69.17	26.43	205
Finland Inarii	FIN12	12	NE	69.07	27.17	157
Finland Kuusamo	FIN24	10	NE	65.81	29.24	269
Latvia, Dunezers Lake	LVA	9	NE	57.3	22.00	19
Estonia Vardi	EST	11	NE	58.3	25.51	77
Russia Yakkima	RUS7	10	NE	61.31	30.08	80
Russia Zoja	RUS8	4	NE	67.41	32.65	163
Hungary Sopron	HUN	15	SE	47.4	16.34	331
Romania Dedulesti	ROM	5	SE	45.01	24.53	444
Serbia Velika Plec	SER	13	SE	44.18	20.03	409
Ukraine Luck	UA1	6	SE	50.75	25.33	243
Poland Pusta Wielka	PuW	10	PLM	49.4	20.82	600
Poland Pieniński National Park	PNP	11	PLM	49.42	20.36	700
Poland Szczeliniec	SZCZ	10	PLM	50.29	16.20	850
Poland Białe skały	BIS	23	PLM	50.46	16.36	755
Poland Dolina Białego	DBS	30	PLM	49.26	19.95	1100
Poland Narożnik	NAR	32	PLM	50.46	16.35	756
Poland Sokolica	SOK	30	PLM	49.42	20.43	730
Poland Tarnawa	TAR	20	PLM	49.06	22.49	660
Poland Wielkie Koryciska	WK	26	PLM	49.26	19.82	1100
Poland Łysa Skałka	TLS	29	PLM	49.15	20.06	1120

Table S2. Summary statistics for mitochondrial DNA (*mt*DNA) and nuclear SNP data. No. – population number; H – number of *mt*DNA haplotypes; Ne – effective number of haplotypes; Rh – haplotypic richness; Hd – haplotype diversity; Ho – observed heterozygosity; He – expected heterozygosity; F_{IS} – inbreeding coefficient.. Population acronyms and geographic regions are listed in Table S1.

Population acronym	No.	*mt*DNA				SNP		
		H	**N_e**	**R_h**	**H_d**	**H_o**	**H_e**	**F_{IS}**
B	**1**	5	2.961	1.785	0.685	0.293	0.294	0.002
Bo	**2**	6	3.879	2.214	0.766	0.294	0.295	0.002
By	**3**	7	3.546	2.076	0.742	0.293	0.294	0.001
Cz	**4**	6	1.948	1.366	0.503	0.293	0.294	0.003
Go	**5**	6	2.445	1.514	0.611	0.293	0.294	0.001
G	**6**	7	4.206	2.370	0.789	0.294	0.294	0.001
H	**7**	7	3.556	2.126	0.746	0.295	0.295	0.000
J	**8**	4	2.739	1.616	0.658	0.294	0.295	0.000
K	**9**	5	2.885	1.778	0.676	0.296	0.295	-0.002
Kb	**10**	7	2.993	1.942	0.690	0.293	0.294	0.001
Md	**11**	3	1.411	0.744	0.301	0.291	0.291	-0.002
Ms	**12**	7	3.710	2.257	0.755	0.294	0.295	0.001
M	**13**	6	3.130	1.961	0.703	0.294	0.294	0.001
Mi	**14**	5	3.193	1.867	0.710	0.295	0.295	0.000
Pi	**15**	5	2.885	1.778	0.676	0.294	0.295	0.002
Pl	**16**	7	2.951	1.854	0.685	0.294	0.295	0.003
Po	**17**	7	2.707	1.900	0.652	0.294	0.295	0.000
Pr	**18**	5	3.483	2.037	0.736	0.294	0.295	0.001
Sp	**19**	7	2.886	1.801	0.675	0.293	0.294	0.002
Ch	**20**	4	1.931	1.147	0.499	0.293	0.294	0.002
L	**21**	4	2.722	1.685	0.655	0.291	0.294	0.007
Su	**22**	5	3.280	1.981	0.718	0.293	0.295	0.003
R	**23**	5	2.563	1.530	0.630	0.294	0.295	0.002
We	**24**	6	2.142	1.476	0.550	0.293	0.294	0.001
Wi	**25**	5	2.651	1.611	0.647	0.294	0.293	-0.002
Wl	**26**	6	2.882	1.759	0.677	0.293	0.294	0.001
Wy	**27**	8	3.571	2.225	0.745	0.294	0.294	0.000
FRA	**28**	2	2.000	0.992	0.556	0.283	0.283	-0.005
CHE	**29**	2	1.220	0.500	0.200	0.285	0.286	0.000
GER	**30**	2	1.220	0.500	0.200	0.294	0.294	-0.003
NO2	**31**	4	2.027	1.403	0.543	0.295	0.295	-0.003
NO3	**32**	4	3.267	2.055	0.747	0.293	0.294	0.001
FIN1	**33**	3	1.412	0.833	0.318	0.301	0.300	-0.008
FIN2	**34**	2	1.198	0.455	0.182	0.300	0.299	-0.005
FIN3	**35**	2	1.180	0.417	0.167	0.299	0.297	-0.009
FIN4	**36**	1	1.000	0.000	0.000	0.301	0.294	-0.024
FIN12	**37**	1	1.000	0.000	0.000	0.295	0.295	-0.007
FIN24	**38**	1	1.000	0.000	0.000	0.300	0.294	-0.018
LVA	**39**	3	2.455	1.667	0.667	0.295	0.296	-0.001
EST	**40**	5	3.571	2.393	0.800	0.296	0.296	-0.005
RUS7	**41**	2	1.471	0.778	0.356	0.299	0.296	-0.014
RUS8	**42**	2	1.385	0.833	0.333	0.296	0.297	-0.009
HUN	**43**	3	2.279	1.319	0.604	0.289	0.291	0.005
ROM	**44**	3	2.273	2.000	0.700	0.293	0.279	-0.056
SER	**45**	1	1.000	0.000	0.000	0.291	0.291	-0.004
UA1	**46**	2	1.923	1.000	0.600	0.296	0.296	-0.026
PuW	**47**	4	2.613	1.944	0.694	0.292	0.287	-0.018
PNP	**48**	1	1.000	0.000	0.000	0.295	0.295	-0.005
SZCZ	**49**	2	1.220	0.500	0.200	0.282	0.286	0.008
BIS	**50**	2	1.403	0.654	0.300	0.293	0.292	-0.003
DBS	**51**	2	1.067	0.161	0.065	0.286	0.275	-0.034
NAR	**52**	6	4.734	2.490	0.815	0.296	0.295	-0.003
SOK	**53**	1	1.000	0.000	0.000	0.295	0.293	-0.008
TAR	**54**	4	1.695	1.101	0.432	0.288	0.287	-0.004
WK	**55**	3	1.235	0.493	0.197	0.276	0.282	0.017
TLS	**56**	4	2.239	1.259	0.572	0.283	0.278	-0.014

4	**2.328**	**1.324**	**0.502**	**0.293**	**0.293**	**-0.004**

Table S3. Model selection for the optimal number of migration edges in TreeMix. Summary of model fits for determining the optimal number of migration edges (*m*) in TreeMix, based on nonlinear curve fitting implemented in the OptM R package. Four models—Piecewise Linear, Bent Cable, Simple Exponential, and Non-linear Least Squares—were compared using Akaike Information Criterion (AIC). The table reports AIC scores, ΔAIC values, and estimated optimal *m* for each model.

Model Type	AIC	ΔAIC	Optimal m
PiecewiseLinear	**–7.80**	**0**	**~1.29**
BentCable	–5.80	2.00	~1.03
SimpleExponential	52.77	60.57	3.00
NonLinearLeastSquares	71.98	79.78	2.00

Table S4. Results of f_3-statistic tests for admixture among regional groups trios. Each row presents the outcome of a three-population test in the form f_3(X; A, B), where X is the target population and A and B are putative source populations. The table reports the f_3-statistic estimate (est), Z-score (Z), and corresponding *p*-value (*p*). Significantly negative f_3 values (Z < –3) provide evidence of historical admixture in the target population.

X	A	B	est	se	Z	*p*
NE	WE	PLM	0.000331	2.18E-05	15.22626	2.37E-52
NE	WE	PL_RSS	0.000466	1.80E-05	25.84833	2.54E-147
NE	SE	WE	0.000544	2.78E-05	19.58234	2.19E-85
NE	PLM	PL_RSS	0.000599	1.54E-05	38.78187	0
NE	SE	PLM	0.000663	2.17E-05	30.51655	1.57E-204
NE	SE	PL_RSS	0.000763	1.90E-05	40.17862	0
PLM	NE	SE	0.000749	3.20E-05	23.43527	1.87E-121
PLM	PL_RSS	NE	0.000813	2.90E-05	28.03907	5.43E-173
PLM	PL_RSS	SE	0.000914	3.25E-05	28.12305	5.12E-174
PLM	PL_RSS	WE	0.000948	3.29E-05	28.80386	1.92E-182
PLM	SE	WE	0.000962	3.88E-05	24.80662	7.60E-136
PLM	NE	WE	0.001081	3.33E-05	32.45558	4.52E-231
PL_RSS	NE	SE	5.83E-05	1.33E-05	4.396897	1.10E-05
PL_RSS	WE	PLM	8.75E-05	1.54E-05	5.68686	1.29E-08
PL_RSS	SE	PLM	0.000122	1.34E-05	9.161117	5.14E-20
PL_RSS	SE	WE	0.000136	2.08E-05	6.524385	6.83E-11
PL_RSS	NE	PLM	0.000223	1.12E-05	19.96176	1.18E-88
PL_RSS	NE	WE	0.000355	1.59E-05	22.28231	5.49E-110
SE	WE	PLM	0.000529	3.49E-05	15.16854	5.71E-52
SE	WE	PL_RSS	0.000564	3.11E-05	18.1194	2.24E-73
SE	PLM	PL_RSS	0.000578	2.73E-05	21.1237	4.82E-99
SE	PL_RSS	NE	0.000642	2.80E-05	22.89966	4.68E-116
SE	PLM	NE	0.000742	3.14E-05	23.65691	1.00E-123

SE	WE	NE	0.000861	3.64E-05	23.63765	1.58E-123
WE	NE	SE	0.000508	3.83E-05	13.27216	3.36E-40
WE	PL_RSS	NE	0.000586	3.31E-05	17.70926	3.56E-70
WE	PLM	NE	0.000721	3.66E-05	19.67814	3.32E-86
WE	PL_RSS	SE	0.000805	3.74E-05	21.54675	5.68E-103
WE	PLM	SE	0.00084	4.12E-05	20.39342	1.91E-92
WE	PLM	PL_RSS	0.000854	3.56E-05	23.95462	8.27E-127

Table S5. Results of f_4-statistic tests for asymmetry in allele sharing among population quartets. Each row presents the outcome of a four-population test in the form f_4(A, B; C, D), where significant deviations from zero indicate a departure from a strictly bifurcating tree model. Estimates (Est), standard errors (SE), Z-scores (Z), and p-values (P) are shown for each test. Significant positive or negative values ($|Z| > 3$) provide evidence of excess allele sharing between one pair of populations relative to the other, consistent with admixture or alternative tree topologies.

A	**B**	**C**	**D**	**est**	**se**	**Z**	***p***
SE	WE	PLM	PL_RSS	3.49E-05	1.92E-05	1.816387	0.069311
SE	PLM	WE	PL_RSS	4.84E-05	2.43E-05	1.991486	0.046427
SE	PL_RSS	WE	PLM	1.35E-05	2.36E-05	0.571188	0.567872
SE	WE	PLM	NE	0.000332	2.45E-05	13.56192	6.73E-42
SE	PLM	WE	NE	0.000213	2.66E-05	7.998536	1.26E-15
SE	NE	WE	PLM	-0.00012	2.83E-05	-4.21654	2.48E-05
SE	WE	PL_RSS	NE	0.000297	1.96E-05	15.15947	6.56E-52
SE	PL_RSS	WE	NE	7.76E-05	2.25E-05	3.454693	0.000551
SE	NE	WE	PL_RSS	-0.00022	2.49E-05	-8.82372	1.11E-18
SE	PLM	PL_RSS	NE	0.000164	1.61E-05	10.20143	1.95E-24
SE	PL_RSS	PLM	NE	6.41E-05	1.66E-05	3.860041	0.000113
SE	NE	PLM	PL_RSS	-0.0001	1.61E-05	-6.21865	5.01E-10
WE	PLM	PL_RSS	NE	-0.00013	1.80E-05	-7.38673	1.50E-13
WE	PL_RSS	PLM	NE	-0.00027	1.95E-05	-13.7115	8.67E-43
WE	NE	PLM	PL_RSS	-0.00014	1.69E-05	-7.96809	1.61E-15

Figure S1. Geographical distribution of the 24 Scots pine (*Pinus sylvestris* L.) seed regions in Poland, each indicated by a distinct colour. Dots mark the sampling locations of individual populations analysed in the study.

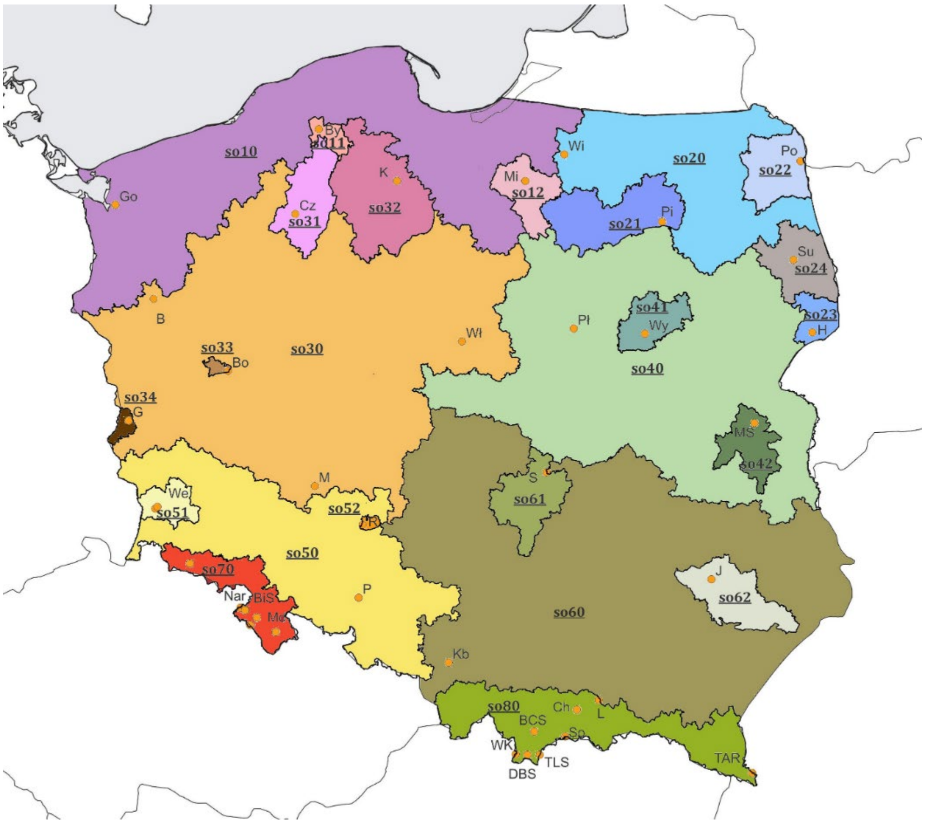


Figure S2. Phylogenetic tree illustrating the relationships between populations based on *mt*DNA genetic distances. The tree was constructed in R and visualized using FigTree.

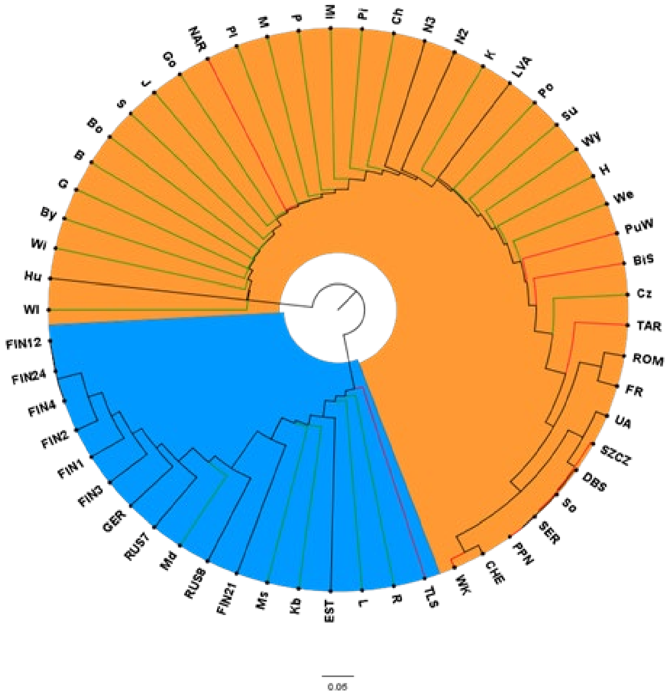


Figure S3. Basic statistic of mitochondrial (*mt*DNA) markers for each of the studied geographical regions; H, number of haplotypes; N_e, effective number of haplotypes; R_h, haplotypic richness; H_d, haplotype diversity.

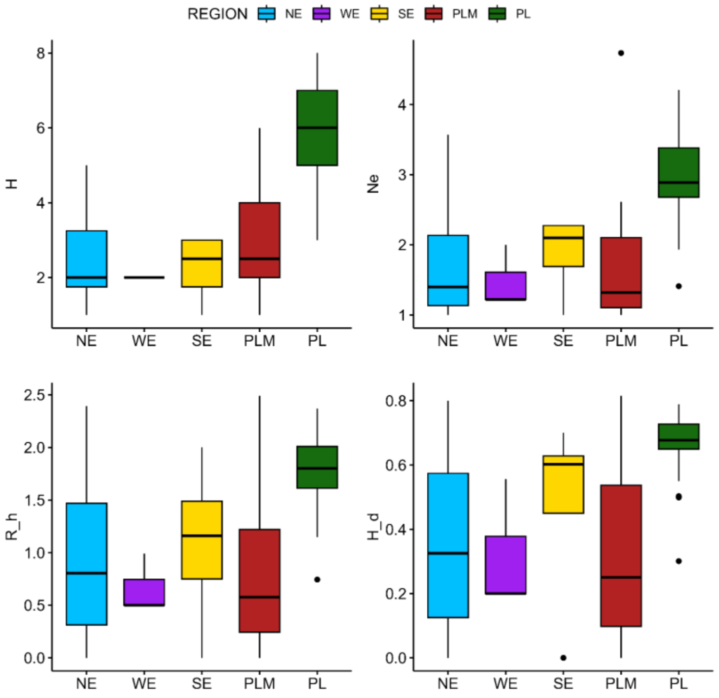


Figure S4. Basic statistics of SNP markers for each of the studied geographical regions.; F_{IS}, averaged inbreeding coefficient; H_o, observed heterozygosity; H_e, expected heterozygosity.

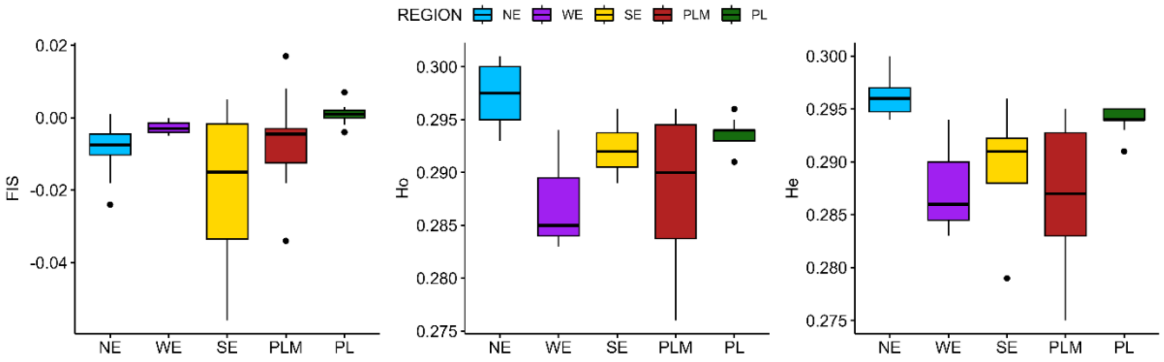


Figure S5. The pairwise F_{ST} heatmap derived from SNP marker data reveals patterns of genetic differentiation among the analyzed geographic regions.

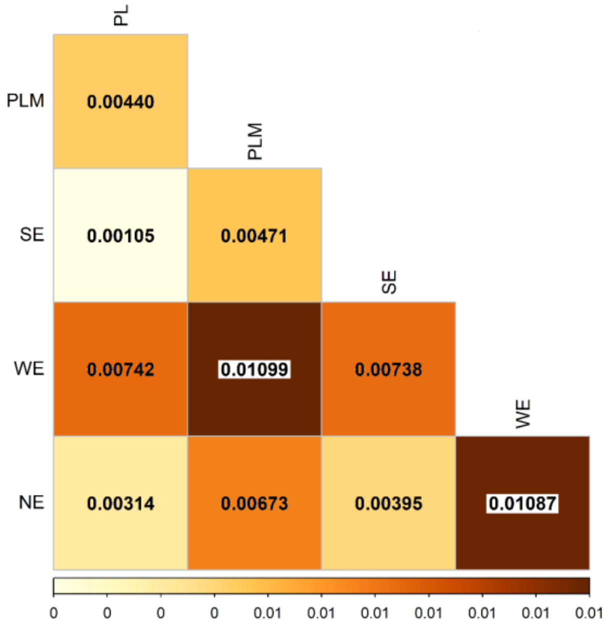


Figure S6. Principal Component Analysis (PCA) based on SNP data performed in R version 4.2. The Polish all mountain populations excluded (a), all populations included (b). Each population is color-coded according to its geographic region, with unique colors assigned to Polish mountain populations for better clarity.

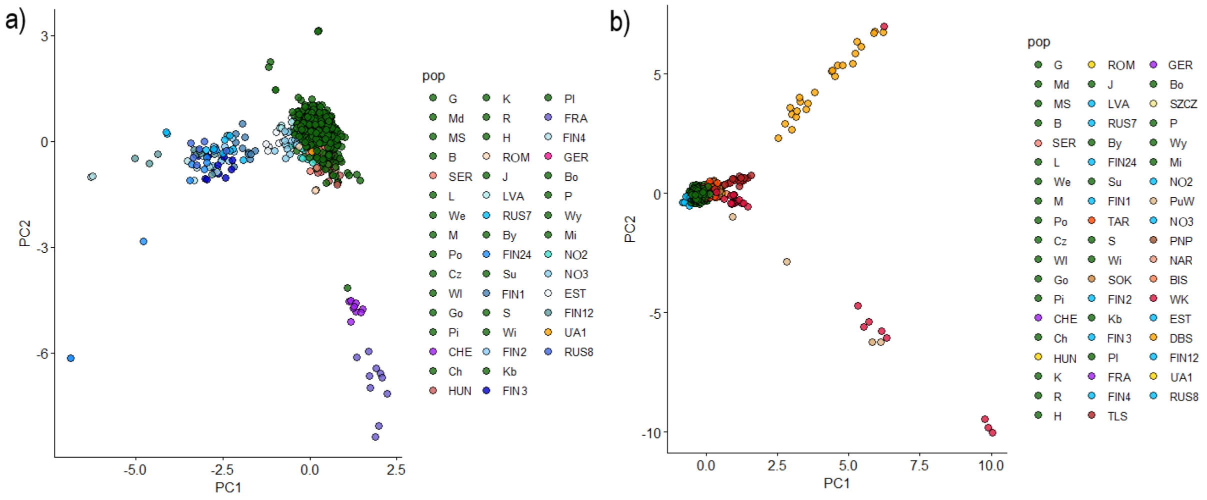


Figure S7. Principal Component Analysis (PCA) based on SNP data performed in R version 4.2. Analysis based on all populations including a subset of the Polish Reserved Seed Stands (a), and with Polish mountain populations excluded (b). Each population is color-coded according to its geographic region, with unique colours assigned to Polish mountain populations for better clarity.

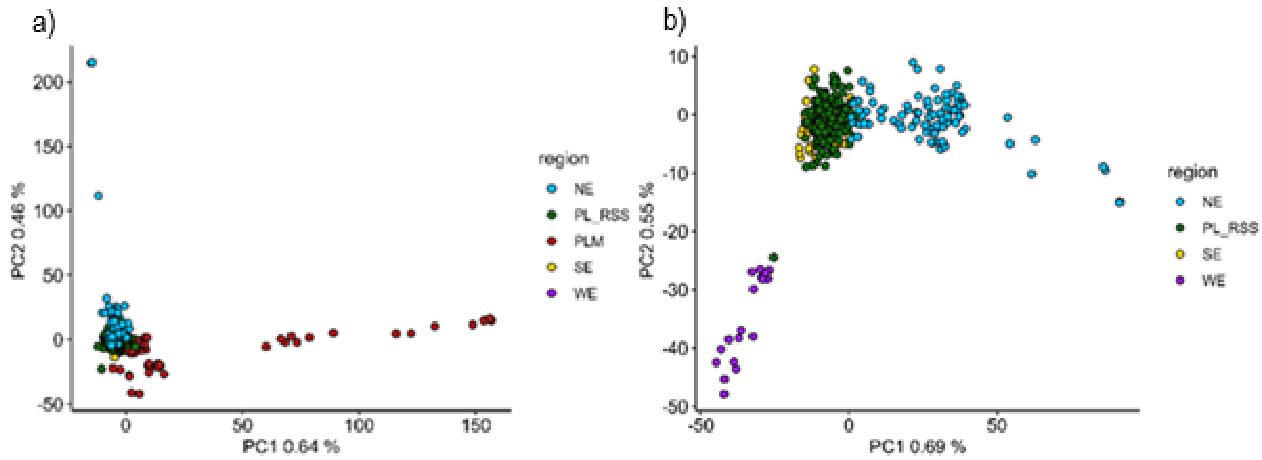


Figure S8. Cross-entropy values for different numbers of ancestral populations (K = 1–10), inferred using the LEA package based on SNP data. The optimal number of genetic clusters was identified at K = 7, corresponding to the lowest cross-entropy value.

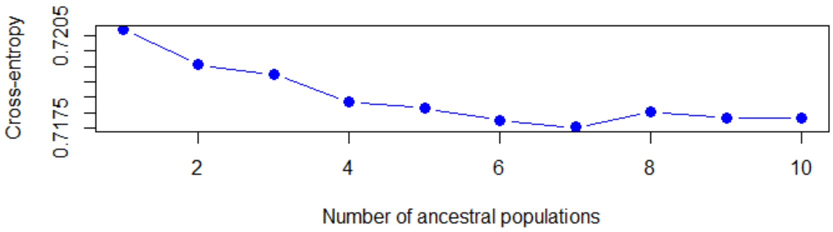


Figure S9. Cross-validation error for regularization parameter in FEEMS. Mean L2 cross-validation error (y-axis) plotted against log10-transformed λ values (x-axis). The vertical orange line indicates λ = 5.45, chosen as the optimal model based on minimum prediction error.

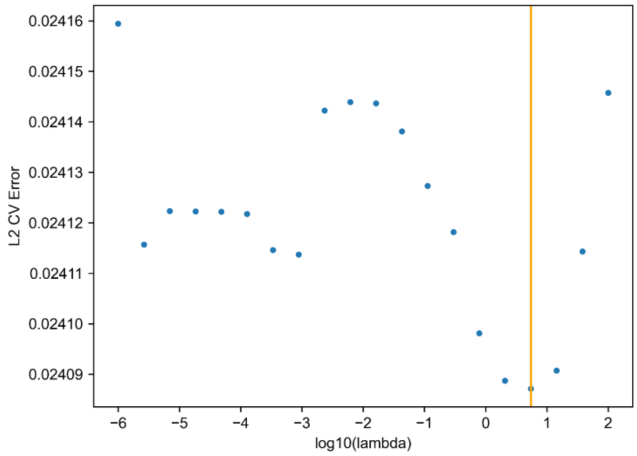


Figure S10. Model fit across migration edges in TreeMix. Log-likelihood values (black circles) plotted for TreeMix models with 0–3 migration edges (m). Curves represent fitted nonlinear models used in OptM; asterisks denote estimated change points. Model selection based on AIC favoured m = 2.

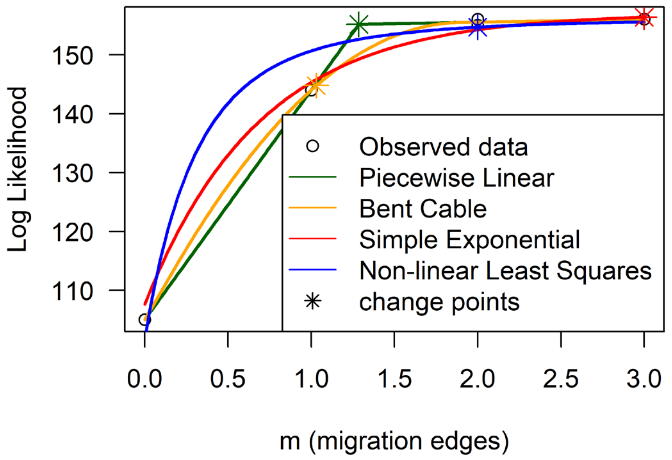
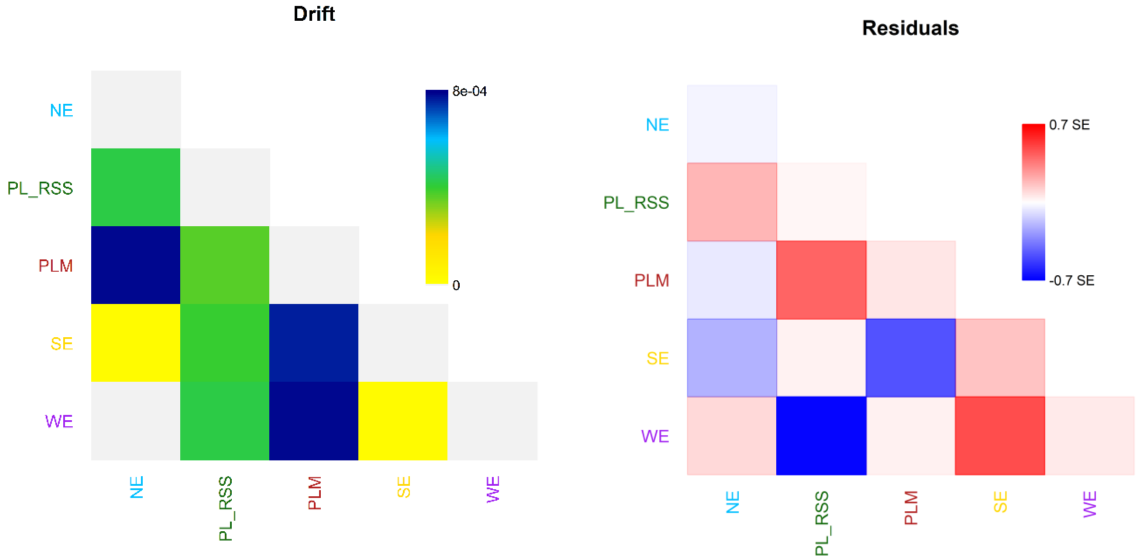


Figure S11. Drift and residual covariance matrices from TreeMix. Pairwise drift matrix (left) and standardized residual covariance matrix (right) derived from the TreeMix model with two migration edges. The drift matrix shows accumulated genetic drift between all pairs of population clusters, colored from yellow (low drift) to dark blue (high drift), scaled to a maximum of 8 × 10^{-4}. The residual matrix displays model fit deviations in standard error units: positive values (red) indicate population pairs more closely related than expected under the fitted tree, while negative values (blue) indicate the opposite.



Rozdział 3

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Balancing Genetic Integrity and Adaptive Potential under Climate Change: The Scots Pine Case

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Abstract:	Scots pine (Pinus sylvestris L.) is one of the most widely distributed tree species in Eurasia and a key component of forest ecosystems in Central and Northern Europe. Understanding phenotypic, climatic, and genetic variation within its populations is essential for sustainable forest management, particularly under the threat of climate change and the increasing frequency of extreme events such as prolonged droughts. In this study, we analyzed 27 populations representing all 24 provenance regions in Poland, including highly valued reserve seed stands (RSS) managed under a strict seed zone regime. Phenotypic traits - tree height and diameter at breast height (DBH) - were measured for 818 dominant trees. Climatic characteristics of the provenance regions were compiled, and genotyping data based on thousands of SNP markers were used to assess the level of neutral genetic differentiation among populations. A linear mixed model was applied to examine the relationships between site index (SI) and climatic factors that potentially influence forest productivity. The results indicate that provenance regions differ significantly in terms of phenotype; however, this differentiation is not reflected in the neutral background of genotypes, nor in regional climatic conditions. Genetic variation showed minimal structure (maximum FST = 0.007), and overall climatic variation across Poland was relatively narrow. The models suggested that aridity (AHM) and continentality (CMD) indices may be particularly relevant for explaining growth performance. These findings suggest that local microclimates, phenotypic plasticity, and biotic interactions jointly shape tree performance. Current strict seed zone regulations, although designed to prevent maladaptation, may inadvertently constrain adaptive potential. A more flexible approach to the transfer of forest reproductive material does not appear to pose a significant risk to Scots pine stands and may instead offer an opportunity to enhance their adaptive capacity and resilience under changing climatic conditions.

Balancing Genetic Integrity and Adaptive Potential under Climate Change: The Scots Pine Case

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Abstract

Scots pine (*Pinus sylvestris* L.) is one of the most widely distributed tree species in Eurasia and a key component of forest ecosystems in Central and Northern Europe. Understanding phenotypic, climatic, and genetic variation within its populations is essential for sustainable forest management, particularly under the threat of climate change and the increasing frequency of extreme events such as prolonged droughts. In this study, we analyzed 27 populations representing all 24 provenance regions in Poland, including highly valued reserve seed stands (RSS) managed under a strict seed zone regime. Phenotypic traits - tree height and diameter at breast height (DBH) - were measured for 818 dominant trees. Climatic characteristics of the provenance regions were compiled, and genotyping data based on thousands of SNP markers were used to assess the level of neutral genetic differentiation among populations. A linear mixed model was applied to examine the relationships between site index (SI) and climatic factors that potentially influence forest productivity. The results indicate that provenance regions differ significantly in terms of phenotype; however, this differentiation is not reflected in the neutral background of genotypes, nor in regional climatic conditions. Genetic variation showed minimal structure (maximum $F_{ST} = 0.007$), and overall climatic variation across Poland was relatively narrow. The models suggested that aridity (AHM) and continentality (CMD) indices may be particularly relevant for explaining growth performance. These findings suggest that local microclimates, phenotypic plasticity, and biotic interactions jointly shape tree performance. Current strict seed zone regulations, although designed to prevent maladaptation, may inadvertently constrain adaptive potential. A more flexible approach to the transfer of forest reproductive material does not appear to pose a significant risk to Scots pine stands and may instead offer an opportunity to enhance their adaptive capacity and resilience under changing climatic conditions.

Keywords: Scots pine, Reserved Seed Stands, Forest Management, SNP genotyping, Phenotypic plasticity, Climate change

1. Introduction

Scots pine (*Pinus sylvestris* L.) rapidly established its dominance across the forests of Eurasia after the Last Glacial Maximum and today possesses the widest distribution of any pine species, extending from the Iberian and Balkan Peninsulas northward to the Barents Sea and from Scotland eastward to the Russian Pacific coast. Within this vast range, the species demonstrates remarkable ecological breadth, thriving in environments from temperate rainforests to arid tundra margins (Houston Durrant et al. 2016). Scots pine is currently one of the most ecologically and economically important tree species in Eurasia, serving both as a dominant forest-forming species and a valuable source of timber. In Poland, it accounts for 58.6% of the total forest area (Zajączkowski et al. 2021). Despite Scots pine’s relatively high drought tolerance (Houston Durrant et al. 2016), ongoing climate change and declining precipitation may lead to productivity loss and forest decline in pine stands. This phenomenon may be particularly significant in regions of Central and Northern Europe, where pine dominates forest ecosystems, resulting in potentially severe economic impacts.

The extensive distribution and pronounced phenotypic variation of Scots pine lead to the description of numerous morphological ecotypes and the establishment of international provenance trials (Giertych and Oleksyn 1992). Long-term studies have shown that Scots pine populations are highly diverse in phenotypic and quantitative traits (Hurme et al. 1997; Perry et al. 2016; Savolainen and Pyhäjärvi 2007; Shutyaev and Giertych 1998), with lowland European provenances - especially from Poland, Germany, Belgium, and the Baltic countries-consistently demonstrating superior growth, high survival, pest resistance, and strong adaptive potential (Kohlstock and Schneck 1992; Giertych 1979). Results from IUFRO trials in Germany confirmed that, alongside local provenances, those from France and Poland performed among the best across sites, whereas drought-adapted Bosnian ecotypes did not (Taeger et al. 2013). The Polish provenances of Miłomłyn, Rychtal, and Bolewice are often described as outstanding in both national and international trials (Barzdajn et al. 2016; Kowalczyk and Wojda 2019; Giertych and Oleksyn 1992; Giertych 1980; Szeligowski et.al. 2016; Szeligowski et al., 2023). Recent comparisons of two IUFRO 1982 plots after 35 years of growth revealed significant variation in productivity and stem form among European origins in central Poland.

In response to the wide variation in phenotypic traits and performance among Scots pine stands, forest managers introduced the concept of regions of origin to protect forest reproductive material (FRM) of exceptional genetic value. In Polish forestry, two classification systems are of particular importance: natural-forest regionalization (Mroczkiewicz 1952; Trampler et al. 1990; Zielony and Kliczkowska, 2012) and seed regionalization, both used to delimitate regions of provenance for FRM (Regulation of the Polish Minister of the Environment of July 29, 2015, on the use of FRM outside the region of origin, Journal Laws of September 8, 2015, item 1328; Matras 2013). These systems play a key role in silvicultural and forest management planning. The seed regionalization framework was designed to mitigate risks from uncontrolled seed transfer, ensuring sustainable use of the seed base, preservation of local gene pools, and high breeding value of forest generations (Matras 2013; Tracz and Zielony 2016). According to the EU regulations, each member state must define provenance regions for every tree species used in reforestation and afforestation programs (Council of the European Union, 1999 Council Directive 1999/105/EC of 22 December 1999 on the marketing of FRM). Poland is currently divided into 24 provenance regions for Scots pine, a system intended to safeguard the genetic integrity and adaptive capacity of local tree populations while promoting their sustainable use. Within a species of such broad distribution, local populations experience distinct environmental pressures that shape adaptive responses (Kawecki and Ebert 2004). Numerous common-garden experiments have documented strong clinal variation and local adaptation in Scots pine (Rehfeldt 1989; Shutaev and Giertych 1998); yet phenotypic plasticity remains a critical factor enabling trees to adjust growth to environmental change (Lande 2009). Despite pronounced phenotypic divergence among provenances, genetic studies have not consistently mirrored these differences. Scots pine exhibits usually high within-population but low among-population genetic diversity (Lasek et al. 2024), and its genetic structure is largely homogenized and panmictic across Eurasia, with differentiation not exceeding 2% (Bruxaux et al. 2024). Such a weak structure, typical of wind-pollinated trees with wide ranges, likely reflects recolonization from multiple refugia and potential human-mediated gene flow (Żukowska et al. 2023).

Although the population genetics of Scots pine has been extensively investigated (Pyhäjärvi et al., 2020), large-scale studies integrating phenotypic, environmental, and genetic variation remain scarce. The present study provides a comprehensive analysis combining climatic, environmental, phenotypic, and nearly 40,000 SNP-based genetic data for 27 populations (818 trees) representing all 24 currently recognized provenance regions in Poland, including maternal trees from Reserved Seed Stands (RSS). By emphasizing the importance of locally sourced reproductive material, this study contributes to a better understanding of phenotypic variation across provenance regions and their relevance for adaptive forest management under changing climatic conditions. The research was designed to compare growth characteristics across all valid seed zones in Poland and to identify potential patterns of climate-related differentiation. We hypothesize that weak selective pressures within the relatively uniform climatic gradient of Poland, combined with local climatic variation and phenotypic plasticity, underlie the observed phenotypic differentiation among Scots pine provenances and influence their growth performance across regions. To test these assumptions, we examine the relationship between the site index (SI) at the reference age of 100 years and selected climatic variables to check whether some climatic factors may directly affect the production performance of Scots pine populations. Moreover, climatic comparisons among populations provide a basis for positioning local populations within the broader European climatic framework, which may contribute to the development of more effective strategies for mitigating climate change–related losses.

2. Materials and Methods

2.1. Study Material, Phenotypic Measurements, and Genotyping

This study included 27 populations representing all regions of provenance in Poland (Table 1). Within the 24 seed regions, the eight main regions correspond to the natural-forest regionalization proposed by Zielony and Kliczkowska (2012); the other 16 regions are all subordinate zones delineated within their boundaries (Supplementary Figure S1). A total of 818 trees from 24 Reserved Seed Stands were selected, with approximately 30 individuals per stand (ranging from 29 to 33). For each tree, the height (H) and diameter at breast height (DBH) were measured, and geographic positions were recorded using a GPS device. For the genetic analysis, we used the dataset previously described by Lasek et al. (2024), based on the PiSy50k SNP array (Affymetrix, Thermo Fisher Scientific, USA). Details of the array design and initial quality assessments are described by Kastally et al. (2022). The array includes polymorphisms derived from transcriptome sequencing and candidate genes resequenced in previous population genetic studies of pine species. Genotyping was performed at Bristol Genomics following DNA amplification, fragmentation, chip hybridization, single-base extension through DNA ligation, and signal amplification according to the Affymetrix Axiom Assay protocol. The procedure was conducted in a 384-well format using the GeneTitan platform (Affymetrix, Thermo Fisher Scientific, USA). Genotype calls were generated using Axiom Analysis Suite software (Applied Biosystems, USA), following the manufacturer's recommendations. After a series of quality control steps, the final dataset comprised 37,597 SNP markers, which were used to calculate summary statistics describing neutral genetic variation across Polish provenances.

2.2. Climatic Data and Environmental Variables

Climatic data representing long-term averages for each stand were obtained from the ClimateEU v.4.63 database for the 1991–2020 period (Marchi et al. 2020) (Table S3). To compare climatic conditions between the tested stands and the entire species range in Poland, data from the CHELSA database (Karger et al. 2017; 2018) were also used. The same database was employed to estimate future climatic conditions for each stand under three scenarios: MPI-SSP126, MPI-SSP370, and MPI-SSP585. These represent different climate pathways: (1) CO_2 emissions reduced to net zero around 2075; (2) emissions remaining constant until 2050 and then declining; and (3) emissions tripling before 2075 (IPCC, 2021).

To reduce multicollinearity, only climatic and stand variables with pairwise correlations $\leq |0.65|$ were used (Table S2). Among the stand characteristics, the following were included: mean diameter of all trees (DBH.all), mean diameter of pine trees (pine.DBH), stand basal area (BA.ha), and the proportion of pine in stand basal area (pine.BA). From the climatic variables, mean annual temperature (MAT), temperature difference (TD), mean annual precipitation (MAP), beginning of the frost-free period (bFFP), and Hargreaves reference evaporation (Eref) were selected. These variables were further used in subsequent analyses, including PCA and linear mixed-effects models.

2.3. Statistical and Spatial Analyses

Spatial patterns of phenotypic traits (H and DBH) and SNP frequencies were examined using QGIS 3.34 “Prizren” software (QGIS Development Team 2023). For phenotypic traits, mean values per population were used, while for genetic data, an F_{ST} matrix was calculated and subjected to principal coordinates analysis (PCoA) with the *cmdscale* function in R. The first PCoA component was visualized as a gradient map. Linear mixed-effects models were applied to assess the influence of environmental factors on phenotypic traits and interpopulation variation, using the *lmer* function in the *lme4* package (Bates et al., 2015). The full model included the following climatic variables: Tave_wt, Tave_sp, Tave_sm, Tave_at, PPT_sp, PPT_sm, PPT_at, MAT, MWMT, MCMT, MAP, MSP, AHM, SHM, DD0, DD5, bFFP, EMT, Eref, CMD, and tree age. After testing several model variants, the best-fitting model was selected based on the lowest Akaike Information Criterion (AIC). For tree height (H), the final model included Tave_wt, Tave_sp, MAT, MWMT, MSP, AHM, EMT, and age. For DBH, the optimal model contained the same variables with the addition of MCMT.

The relationship between the SI index and particular variables was analyzed using a linear mixed model with the modified *lmer* function. The model took into account the linear and quadratic effects of the variable, the main effect of the region, and the interaction between the variable and the region. In order to account for spatial variability between sites, a random capture effect was applied to the Site variable. The final form of the model had the following structure: SI ~ Variable + I(Variable^2) + Region + Variable: Region + (1 | Site). Results were visualized with *ggplot2* in R (v4.2; Wickham 2016). The efficiency of the Site Index was assessed under varying climatic conditions across eight natural forest delineation regions (Figure S1).

2.4. Similarity Analyses and Statistical Tests

The Mantel test (*mantel* function from *vegan* package; Oksanen et al. 2025) was applied to assess correlations between individual dissimilarity matrices (Figure S7). Differences in phenotypic traits among populations were tested using ANOVA followed by a post-hoc Tukey's HSD test implemented in the *agricolae* package in R (de Mendiburu and Yassen 2020). To assess the similarity between populations, dissimilarity matrices were calculated and visualized as clustering heatmaps using the pheatmap function from the *pheatmap* package (Kolde 2025). Separate matrices were created for phenotypic data (Figure S8A), climatic data based on variables used in the mixed-effects model (Figure S8B), spatial data based on Euclidean distances between populations (Figure S8C), and genetic data represented by F_{ST} values (Figure S8D).

3. Results

3.1. Stand Structure and Phenotypic Variation

In most of the sampled stands, Scots pine was the dominant species in the overstory. However, all stands exhibited complex spatial structures and species compositions, with pine comprising the major component of stand basal area in most, though not all, cases (Supplementary Figure S2, S3; Supplementary Table S1). In several stands, the diameter distributions indicated an ongoing change in species composition toward increased contribution of broadleaved or other coniferous species such as beech, oak, or spruce (Supplementary Figures S2-S4; Supplementary Table S1). Substantial differences in tree diameters and heights were observed among the sampled stands (Figure 1; Supplementary Figure S3). Although this comparison includes both environmental and genetic effects, as well as age variation, the overall range of values demonstrates significant differences in pine productivity among the examined populations. The heights of dominant trees, forming the basis of the site index (SI), also varied notably between populations. Because only dominant trees were sampled and the variation was not clearly associated with forest site types (Figure 1), part of the observed differences likely have a genetic component. The analysis of variance (ANOVA) followed by the HSD post-hoc test confirmed statistically significant differences in height (H) among populations (Supplementary Figure S9). The tallest trees were recorded in Hajnówka (mean H = 37.97 m, SD = 2.19), while the shortest were observed in Włocławek (mean H = 25.41 m, SD = 4.00). The largest DBH was also found in Hajnówka (75.01 cm, SD = 7.89), whereas the smallest occurred in Janów Lubelski (44.33 cm, SD = 7.02). A detailed summary of sampled trees and stand characteristics is provided in Table 1, Table S1, and Table S5.

3.2. Climatic Relationships and PCA Analysis

Few significant relationships were identified between stand structural characteristics and climatic variables, with the strongest correlation indicating that greater site productivity (basal area) was associated with higher climatic water availability (Supplementary Table S2). Principal component analysis (PCA) based on stand and climatic variables (Figure 2D; Supplementary Table S3) revealed two partially overlapping groups of populations, with the first two components explaining 51% of the total variance. The first group comprised western and southwestern populations located at sites with lower continentality (TD) and higher mean annual temperature (MAT), while the second group included the remaining populations. Highland populations such as Międzylesie, Stary Sącz–Lipnica, and Stary Sącz–Chełmiec also clustered with the latter group, occupying sites characterized by shorter frost-free periods and higher precipitation (Figure 2D; Supplementary Table S3). Two ecoplots illustrated the climatic position of the tested populations in relation to the species' range. The first showed that Polish populations cover a wide tolerance range of mean annual temperature (MAT) and mean annual precipitation (MAP), with some located near the upper thermal values of Scots pine occurrence in central Europe (Fig. 3A). The second compared the current and projected future conditions under three SSP scenarios (Fig. 3A). Most stands are expected to experience a significant temperature increase with a slight rise in precipitation, while the Kobiór stand already occurs under climatic conditions similar to those projected for other stands in the SSP126 future scenario (Supplementary Figure S5).

3.3. Spatial and Genetic Patterns

Gradient maps revealed a clear spatial differentiation in tree height, with northeastern populations showing generally higher values compared to the rest of the country (Figure 2A). In contrast, no consistent spatial pattern was detected for DBH (Figure 2B). The genetic differentiation based on the F_{ST} distance matrix derived from SNP data showed distinct spatial trends. The first component from the PCoA analysis (Figure 2C) indicated that the Międzylesie population was clearly distinct from the others. A west–east gradient in the PCoA plot suggested that geographic distance influenced genetic similarity among populations. The dissimilarity matrices further supported these patterns (Supplementary Figure S8). Phenotypic dissimilarity highlighted the distinctiveness of the Hajnówka–Spała pair (Supplementary Figure S8A), while climatic dissimilarity separated mountain stands from lowland populations (Supplementary Figure S8B). The F_{ST} matrix confirmed the distinct position of the Międzylesie population (Supplementary Figure S8D). Mantel tests revealed that correlations between most matrices were not significant, except for the F_{ST}–Euclidean distance pair (Mantel $r = 0.254$, p

$= 1 \times 10^{-4}$; Fig S7). The F_{ST}–climate correlation was near the significance threshold (Mantel r = 0.2496, p = 0.0844).

3.4. Environmental Drivers of Phenotypic Variation and Site Productivity

The linear mixed-effects model identified several climatic predictors with significant effects on tree height (H): mean winter temperature (Tave_wt), mean warmest month temperature (MWMT), mean summer precipitation (MSP), annual heat–moisture index (AHM), and extreme minimum temperature (EMT) (Supplementary Figure S5). Higher values of AHM and MSP were associated with reduced height, while increasing EMT also corresponded to shorter trees. In contrast, Tave_wt and MWMT had positive relationships with tree height. For DBH, higher MWMT, Tave_wt, and tree age were associated with larger diameters, whereas higher MAT was negatively related to DBH (Supplementary Figure S5). The mixed model for SI (Figure 4) evaluated climate-driven variation in site productivity (Site Index) across eight natural-forest delineation regions. Among the tested variables, only AHM and climatic moisture deficit (CMD) were statistically significant ($p < 0.05$). The results indicated that Scots pine in regions So8_ and So5_ required higher CMD values for optimal growth, while populations from the remaining regions responded more uniformly. The So2 region maintained the optimal site index values under slightly reduced AHM.

Discussion

4.1. Phenotypic Variation and Stand Structure

The presented study provided a comprehensive assessment of environmental conditions, phenotypic variation, and genetic background across 27 Scots pine populations representing all regions of provenance in Poland. Despite the generally panmictic genetic structure with high within-population and low among-population differentiation (Lasek et al. 2024), a pronounced phenotypic variability in tree height and diameter at breast height (DBH) was observed between stands. The difference between the poorest- and best-performing provenances reached 12.6 m in mean height, and 30.7 cm for DBH, indicating substantial divergence in growth potential even among populations selected as superior sources of forest reproductive material (FRM). The best-performing populations in terms of height were Hajnówka, Wichrowo, and Pomorze, all located in northeastern Poland. Because of their geographical proximity, these populations appear to correspond to the “Baltic” (or “Riga”) pine ecotype, which has been described as exhibiting exceptional wood quality (Daszkiewicz and Oleksyn 2005; Giertych, 1979). In terms of DBH, the highest values were recorded in Hajnówka, Spała, and Kaliska, with Hajnówka ranking first for both traits. Notably, certain populations exhibited high values of tree height but relatively small diameters (e.g., Włocławek, Supraśl), while others showed the opposite trend (e.g., Bytów, Pomorze). Such variation was not associated with forest type and appeared both in conifer-dominated and mixed stands, although mixed stands tended to show slightly higher mean growth values, supporting the growing practice of promoting structurally and compositionally diverse forest ecosystems. Significant differences in other pine phenotypic traits - relative wood density - have been earlier described for Polish provenances, despite relatively small distances between particular stands (Szaban et al., 2023).

4.2. Provenance Performance within a Climatic Context

All tested RSS stands were classified as superior and designated as sources of local forest reproductive material (FRM); however, their growth performance differed markedly. Similar observations have been reported from numerous “common garden” experiments, where a single “leading provenance” could not be unequivocally identified (Barzdajn et al. 2016; Chmura, 2000; Chmura et al. 2003; Matras 1989). The progeny of some of the best-performing provenances, such as Bolewice, appear to be unstable, exhibiting significantly reduced growth when established in unsuitable habitats. For example, Szeligowski et al. (2023) observed considerable variation in mean diameter at breast height (DBH), tree height (H), and stand volume (V) at a 50-year-old study plot in central Poland. In terms of mean DBH and height (H), Bolewice ranked highest, followed by Rogów and Karsko. Similarly, the exceptional performance of Bolewice, Miłomłyn, and Rychtal provenances has been repeatedly reported in the literature and IUFRO trials (Szeligowski et al. 2023; Kowalczyk 2019; Remlein et al. 2015; Giertych 1980; Giertych 1995).

In contrast, in a 50-year-old trial plot in the Carpathian Mountains (Hebda and Wachowiak, 2019), the Bolewice provenance ranked among the poorest performers, placing second lowest for height growth and third lowest for DBH. The highest mean DBH and tree height were recorded for the Tabórz (So12) population, corresponding to our Miłomłyn site. The well-known Rychtal site exhibited moderate growth but relatively low DBH. The authors also noted large phenotypic variation among the tested provenances. The poor performance of provenances that typically grow well elsewhere suggests a strong influence of local microclimatic conditions. These may include water availability, the onset of the frost period, or mean annual temperature, which can affect the growth of specific morphotypes.

Although climatic variation among Polish provenances is relatively small compared to the species' full European range, even subtle differences in local site conditions can lead to marked phenotypic differentiation. Across Poland, the mean annual temperature ranges from 6.8 °C in the mountains to 9.6 °C in the western lowlands, while mean annual precipitation remains relatively uniform except for the southern So70 and So80 delineation regions. Climatic and phenotypic models suggest that only a few tested climatic variables significantly influence

Scots pine growth performance. Furthermore, multivariate analyses revealed a clear division of RSS populations into two geographical groups: (A) western and southwestern populations, located in areas with lower continentality and higher mean annual temperature, and (B) the remaining populations, including highland plots, situated in regions characterized by shorter frost-free periods and higher precipitation. The overall narrow climatic envelope within Poland reinforces the view that climate alone is unlikely to explain the magnitude of phenotypic variation observed among provenances, while the identified groupings point to contrasting thermal and moisture regimes that may differentially filter provenance performance. Earlier studies demonstrate that Scots pine shows considerable variation in its hydraulic architecture depending on the climate of its site of origin (Semerci et al. 2017). As demonstrated by Matisons et al. (2019), the wood anatomy suggests that in the Northern hemisphere, the top-performing Scots pine provenances show low climatic sensitivity, high resilience to weather extremes, and strong adaptive potential, making them suitable for breeding and commercial forestry. Nonetheless, the climatic data confirm that the examined seed regions differ markedly, which may affect the adaptive capacity of the FRM originating from them. For example, the results from the provenance experiment in the Carpathian Mountains illustrate that provenances such as Bolewice and Rychtal, often ranking among the best performers under lowland conditions, performed poorly at higher elevations (Hebda et al., 2017). In contrast, northeastern provenances (Hajnówka, Miłomłyn, Pomorze) originating from colder and wetter climates maintained high performance under harsher and more humid conditions (Hebda et al., 2017). This pattern suggests differential local adaptation to temperature and precipitation regimes rather than a simple latitudinal gradient.

4.3. Genetic Structure and Its Relationship to Phenotype

Neutral genetic variation among Polish RSS populations was very low (maximum $F_{ST} = 0.007$ in Międzylesie So70), which is consistent with generally low between-population genetic variation in Scots pine (Bruxaux et al. 2024). Despite the weak overall genetic structure, a gradual F_{ST} gradient was observed across Poland, with the lowest values occurring in the east and increasing towards the west. This genetic pattern does not correspond to the overall growth performance of the provenances. Even within a largely panmictic genetic structure, substantial phenotypic differentiation was observed among stands, implying that local adaptation or phenotypic plasticity underlies much of the observed variation in growth traits. Comparable discrepancies between genetic and phenotypic variation have also been reported for frost-hardiness traits in Scandinavian Scots pine populations (Hall et al. 2021). Studies of populations from the southern Baltic coast have demonstrated the influence of weather factors on heritable phenotypic traits. The highest heritability was estimated for the sensitivity of radial growth to the summer moisture regime, as well as to conditions prevailing in the winter and late summer of the previous year (Matisons et al. 2019).

At this stage, the observed phenotypic variability cannot be regarded as evidence of local climatic adaptation without further analyses of potentially adaptive SNP outliers and progeny tests, which are planned for future research. Nevertheless, some climatic variables were found to significantly influence DBH and H, as indicated by the linear mixed-effects model. However, given the potential influence of multiple interacting abiotic and biotic factors – such as within-stand competition, pathogen pressure, soil heterogeneity, and microclimatic variation – the observed associations should not be interpreted as direct or causal climate–phenotype relationships.

4.4. Climate Change Implications and Adaptive Potential

Effective management of Scots pine, one of the most economically important timber species in Europe, is becoming increasingly challenging under ongoing environmental change. These changes are already affecting forest productivity through reduced tree fitness, drought stress, pathogen outbreaks, and increased mortality across the species' natural range. Results from modeling analyses (Dyderski et al. 2018; 2025) and climatic comparisons suggest that many Polish stands may face serious stress before the end of the 21st century. Decreased fitness and vitality of pine populations have already been observed across Central and Western Europe (Brichta et al. 2024), primarily due to rising temperatures. However, prolonged drought periods appear to be an even more critical factor for this species, as Scots pine performs best under stable rainfall regimes. Water availability during the warmest part of the year is particularly important, since prolonged droughts, exacerbated by high temperatures, can cause extensive dieback and decline in productivity. Another factor directly linked to water stress is the increasing occurrence of mistletoe (*Viscum album* subsp. *austriacum*), which has become a major problem in Central Europe (Walas et al. 2022). Therefore, the combination of unfavorable climatic trends and biotic stressors may threaten Scots pine forests that dominate large areas of Central and Eastern Europe.

According to a literature review on environmental adaptive drivers (Hallingbäck et al. 2021), the growth performance of Scots pine populations across their European distribution is primarily determined by drought intensity and mean temperature. In the southern range margin, elevated summer temperatures and drought constitute the key limiting factors, whereas in the northern margin, growth performance is influenced by multiple factors such as drought, growing-season temperature, photoperiod, and spring temperature. Dendrochronological analyses of four European origin populations indicated that temperature constitutes the predominant factor controlling radial growth, except in Great Britain, where both precipitation and temperature exerted comparable, moderate effects (Brichta et al. 2024). On the other hand, Vizcaíno-Palomar et al. (2019) suggest that the Spanish Scots pine populations are locally adapted to drought. The strong adaptive plasticity observed in European provenances of native coniferous species may serve as a valuable foundation for creating forests resilient to climate change. As described by Chen et al. (2019), using examples of spruce plots planted across Sweden, provenances from southern locations (such as Poland or Romania) often outperformed local material in terms of growth rate, despite a later onset of the growing season. It is reasonable to assume that the Central European lowland origins of Scots pine would likely be a promising source of propagation material for assisted migration programs (Milesi et al. 2019; Savolainen et al., 2013). Collecting such data is very important from the point of view of forest management. The assisted migration approach should be planned with caution and preceded by collecting data from provenance trials – sometimes provenances that are exceptionally resistant to a given environmental factor within their natural range may have lower fitness in other conditions. For example, at a research site in Germany, the drought-resistant provenances from Bosnia were less suitable than local provenances, as well as French and Polish ones (Taeger et al. 2013). The mixed models for SI identified two climatic variables – annual heat–moisture index (AHM) and climatic moisture deficit (CMD) – as the most significant predictors of site productivity. Scots pine populations from the western and southwestern regions performed best under higher AHM and CMD conditions, whereas the mountain populations from region So8 required greater water availability and moderate temperatures for optimal growth. These findings underscore the importance of region-specific forest management strategies that account for climatic limitations and highlight the need to consider both temperature and water balance when selecting reproductive material for reforestation and breeding programs.

Despite these challenges, Polish Scots pine populations exhibit high phenotypic diversity, substantial genetic variation, and pronounced adaptive plasticity. Recent landscape-genomics work across 39 natural populations identified fine-scale SNP–SNP-environment associations and selection signatures tightly correlated with mean annual temperature, indicating standing adaptive variation relevant to future climates (Łabiszak and Wachowiak 2025). At the same time, genomic-offset projections to 2050 suggest large spatial mismatches between current allelic frequencies and those optimal under projected climates, with natural selection alone likely requiring centuries to close this gap; thus, exposure and sensitivity components of vulnerability may be substantial (Łabiszak and Wachowiak, 2025). Although ecological niche models (Dyderski et al. 2025) predict unfavorable conditions for Scots pine in Central Europe by the end of the century, these projections do not capture evolutionary adaptability. Empirical evidence shows that Scots pine can increase drought tolerance through local adaptation and selection (Vizcaíno-Palomar et al., 2019), and in a provenance study at the southern range margin in Turkey, artificial stands of *P. sylvestris* were able to thrive under warm, relatively dry conditions, with survival rates between 35% and 55% after 13 years (Gülcü & Bilir, 2017). Taken together, these findings indicate that pessimistic climate scenarios and more frequent extremes (prolonged droughts, strong winds, floods) pose real risks to Scots pine stands. Conversely, ecological niche modelling (Supplementary Materials, Figure S6) indicates that some populations—particularly those in high-elevation stands—are projected to experience substantially higher annual precipitation than at present. Interestingly, increased precipitation is expected for both of the tested climatic scenarios. Although overall water availability is expected to increase across the tested areas, the rainfall will likely occur in rapid and intense events. Such shifts in precipitation patterns can alter catchment-scale water distribution, reducing soil water retention while enhancing runoff and reservoir inflow (Eekhout et al., 2018). Consequently, plants may experience elevated physiological stress, especially during summer, despite the higher annual precipitation. Nevertheless, the adaptive capacity of Polish provenances – potentially augmented by carefully validated forest-assisted migration and targeted selection of source populations – may mitigate adverse impacts and enable populations to persist under future environmental conditions, even as forest composition and structure change (Łabiszak and Wachowiak 2025).

Conclusions

Polish Scots pine provenances exhibit substantial phenotypic differentiation that cannot be explained solely by neutral genetic structure or climatic gradients. Growth performance appears to be shaped by the combined effects of multiple biotic and abiotic factors, as well as potentially adaptive genetic variation not captured by neutral markers. Given the limited climatic heterogeneity within Poland, strict provenance zoning may inadvertently constrain adaptive responses under future climate scenarios. Further genomic studies focusing on adaptive SNPs, coupled with progeny and assisted migration trials, are essential to clarify the genetic basis of observed phenotypic variation. Because Polish stands occupy a transitional climatic zone characterized by relatively warm and dry conditions within the species' European range, they may serve as valuable FRM sources for assisted migration and breeding programs. The considerable phenotypic plasticity and apparent adaptive potential of Polish Scots pine populations provide cautious optimism for their continued role in Central European forestry under changing environmental conditions.

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CRediT authorship contribution statement

Martyna Lasek: Conceptualization, Writing- Original Draft, Formal Analysis, Methodology, Writing - Review & Editing, Investigation, Validation, Data acquisition. **Łukasz Walas**:

Formal Analysis, Methodology, Software, Writing - Review & Editing, Visualization. **Daniel Chmura**: Data acquisition, Formal Analysis, Methodology, Writing - Review & Editing.**Witold Wachowiak**: Conceptualization, Funding Acquisition, Data acquisition, Writing - Review & Editing, Writing- Original Draft, Supervision.

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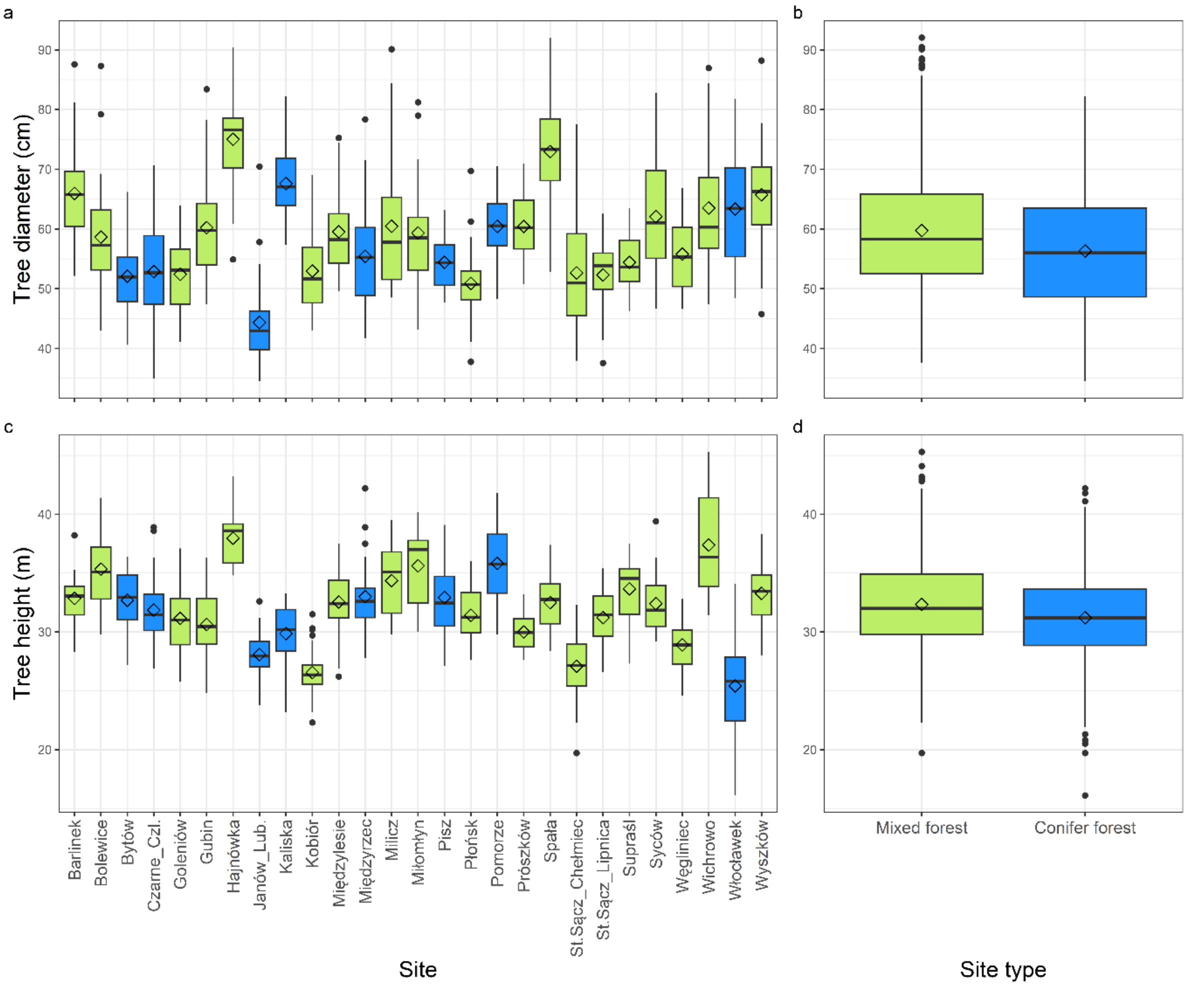

Fig. 1. A boxplot showing variation of diameters and heights of pine trees among examined stands (a, c), and between two forest site types (b, d). A horizontal bar within the boxplot indicates the median, and a diamond shows the mean value.

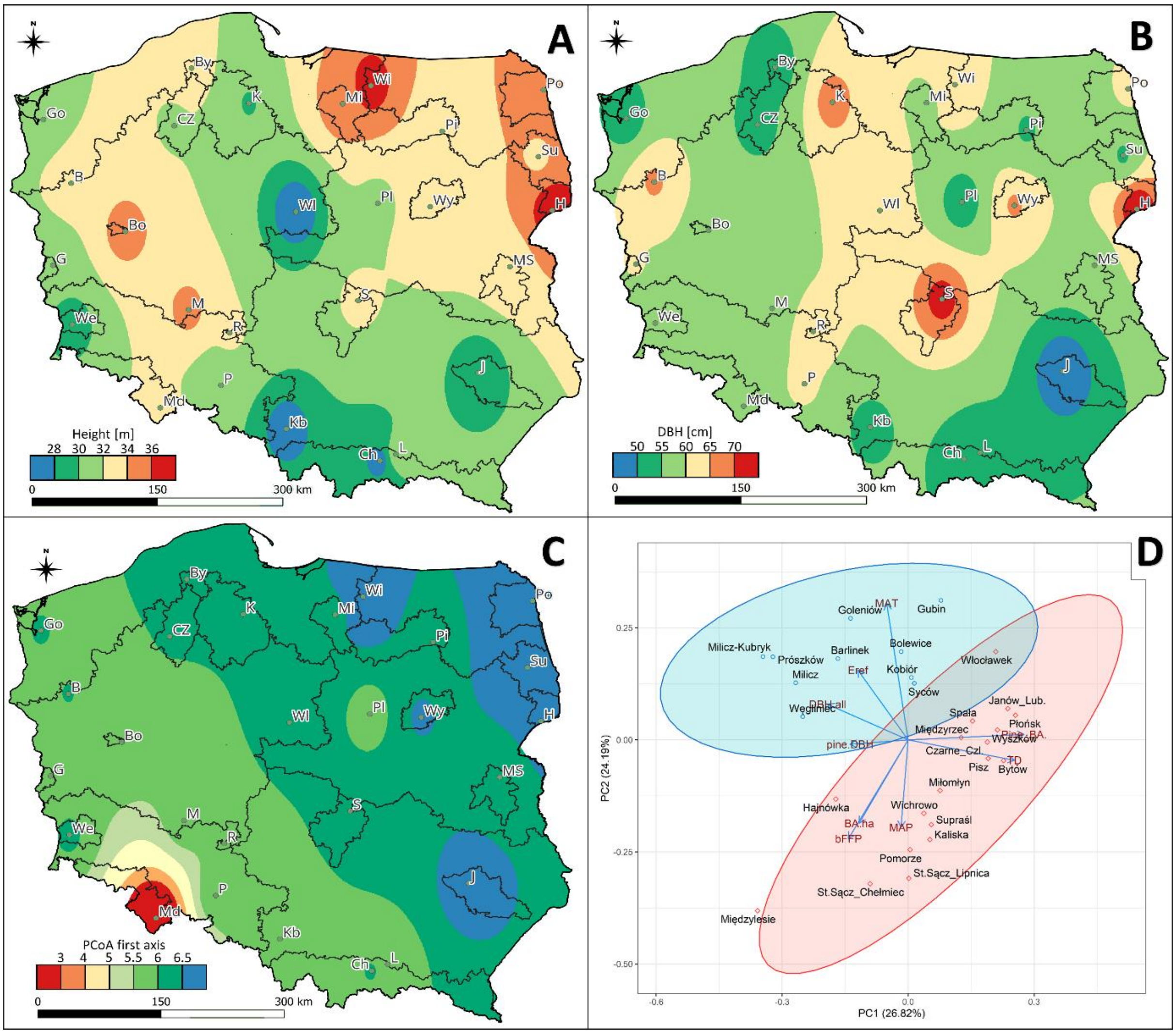

Fig 2. Comparison between the studied populations. A - gradient of the tree height; B - gradient of the DBH; C – genetic gradient according to the PCoA (first component) on the F_{ST}; D - the PCA analysis graph showing the ordination of sampled pine populations according to the first two PCs explaining 51% of variation based on stand and climate variables. Black contour lines indicate the boundaries of seed regions on the maps. In D), a group of western and southwestern populations is marked with blue circles, and the remaining ones with red diamonds. The confidence ellipses are drawn assuming a multivariate normal distribution.

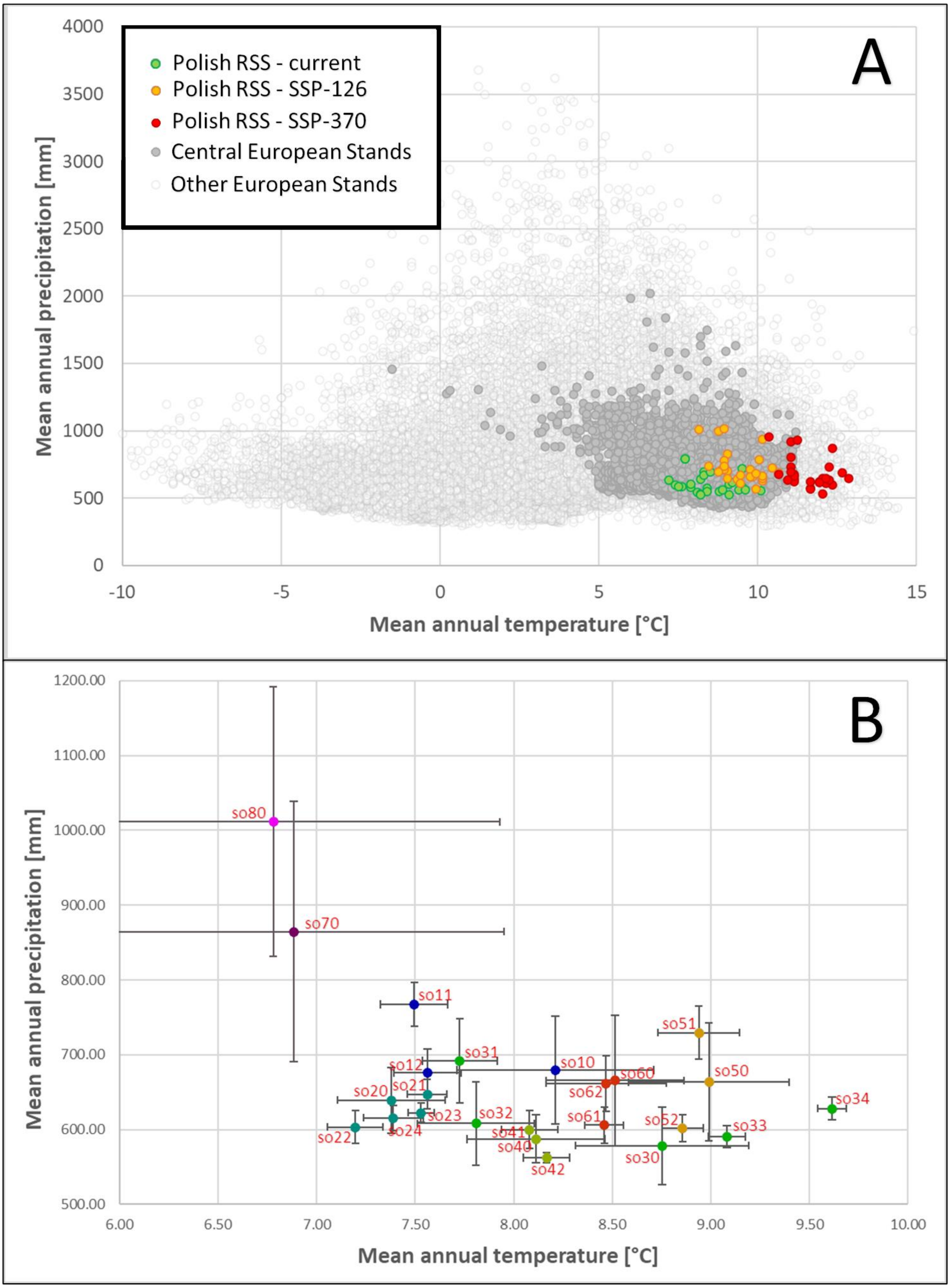

Fig. 3. (A) The Ecoplot displays population locations across the European range of Scots pine, positioning them according to mean annual precipitation (MAP, y-axis) and mean annual temperature (MAT, x-axis). Populations are color-coded as follows: orange – Polish RSS, dark gray – Central European Scots pine, and light gray – populations across the entire European range. (B) The Ecoplot analysis on climatic data among the provenance regions (So).

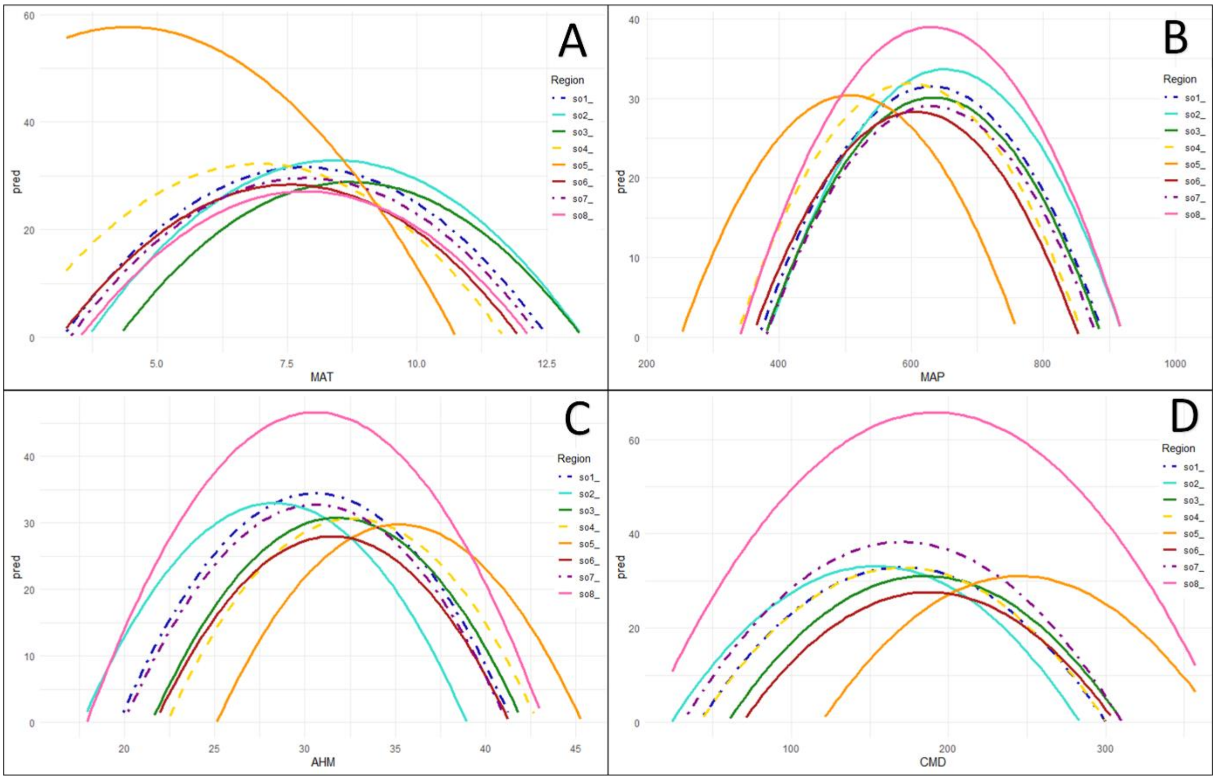

Fig. 4. The mixed linear model applied across eight major natural forest delineation regions (So). The model correlates the Site Index (SI) with key climatic variables, including: A) Mean Annual Temperature (MAT); B) Mean Annual Precipitation (MAP); C) Annual Heat-Moisture Index (AHM), and D) Climate Moisture Deficit (CMD). Among tested, the correlations with AHM and CMD were only statistically significant ($p < 0.05$).

Population (Acronym, RP*)	N	Age	Elevation (m a.s.l)	DBH (cm)	H (m)	SI (m)
Barlinek (B, So30)	30	188	100.62	65.95	32.85	28.88
Bolewice (Bo, So33)	36	150	102.96	58.64	35.33	32.40
Bytów (By, So11)	31	146	120.76	52.07	32.69	29.98
Czarne (Cz, So31	30	154	145.55	52.86	31.86	28.88
Goleniów (Go, So10)	31	176	18.17	52.39	31.15	27.53
Gubin (G,So34)	32	199	71.33	60.21	30.63	26.52
Hajnówka (H, So23)	30	171	179.74	75.01	37.97	34.27
Janów_Lub. (J, So62)	30	124	223.54	44.33	28.07	26.48
Kaliska (K, So32)	30	215	125.18	67.60	29.86	25.50
Kobiór (Kb, So60)	30	186	273.50	52.96	26.55	22.89
Międzylesie (Md, So70)	33	171	547.42	59.52	32.54	29.01
Międzyrzec (Ms, So42)	32	144	164.22	55.42	32.99	30.35
Milicz (M, So30)	32	155	197.91	57.82	34.93	31.82
Miłomłyn (Mi, So12)	30	162	146.78	59.32	35.62	32.26
Pisz (Pi, So21)	30	158	135.99	54.41	32.93	29.78
Płońsk (Pl, So40)	30	110	130.68	50.88	31.41	30.63
Pomorze (Po, So20)	32	151	49.52	60.43	35.82	32.84
Prószków (P, So50)	32	188	207.67	60.42	29.99	26.14
Spała (S, So61)	31	204	200.89	72.96	32.49	28.21
Chełmiec (Ch, So80)	32	125	532.27	52.65	27.08	25.46
Lipnica (L, So80)	30	140	502.78	52.30	31.19	28.76
Supraśl (Su, So24)	31	155	175.53	54.41	33.66	30.59
Rychtal (R, So52)	34	166	216.70	62.07	32.41	29.02
Węgliniec (We, So51)	32	188	200.82	55.78	28.90	25.09
Wichrowo (Wi, So20)	30	182	109.77	63.51	37.39	33.41
Włocławek (Wl, So30)	30	188	69.49	63.33	25.41	21.77
Wyszków (Wy, So41)	30	184	128.66	65.72	33.28	29.39

Table 1. Characteristics of the sampled trees from the studied Scots pine populations: RP – Region of Provenance, N-number of individuals in populations, mean age of populations, elevation, DBH-Diameter at breast height, H- tree height, SI – site index averaged for the age of 100 years.

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Witold Wachowiak reports financial support was provided by National Science Centre Poland. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix

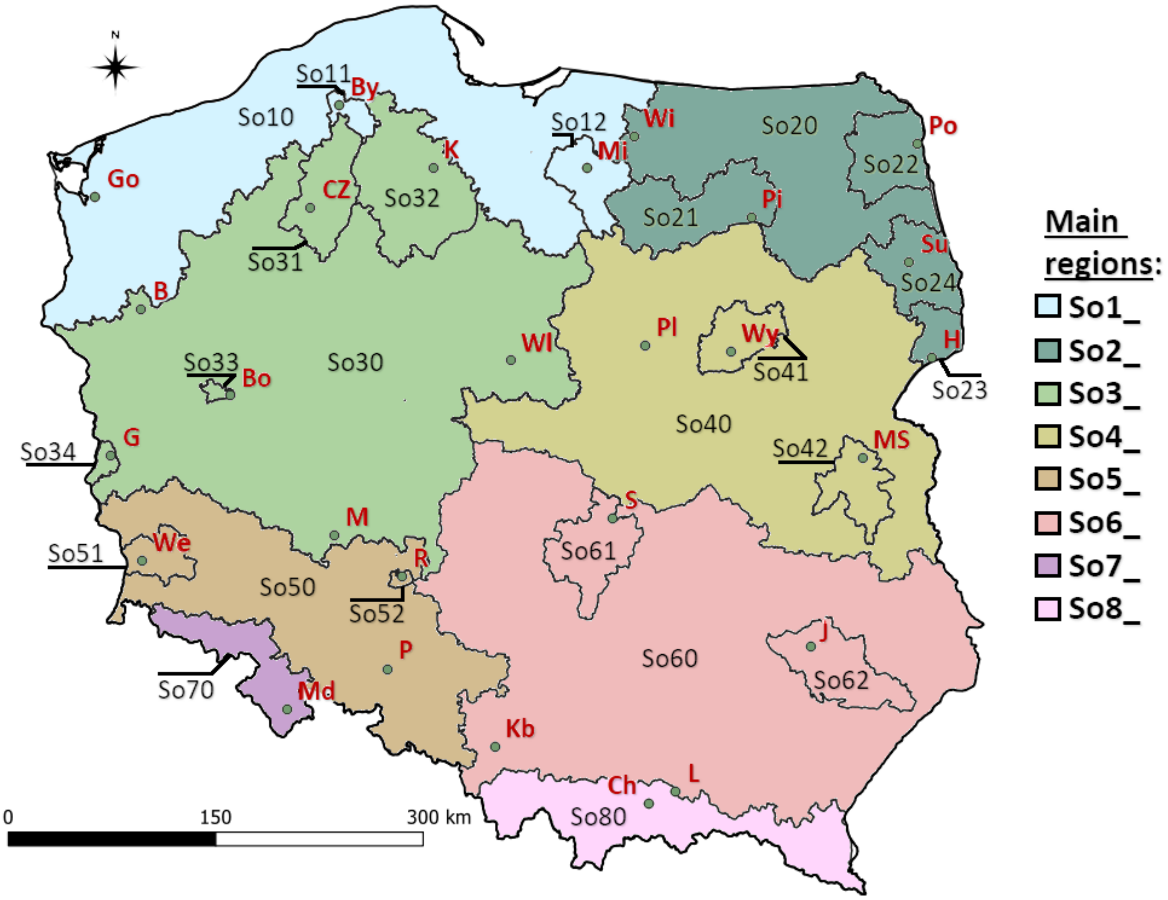

Fig. S1 – Map showing the distribution of 24 Scots pine seed regions in Poland, located within eight natural forest regions. Each main natural forest region is highlighted with a distinct colour.

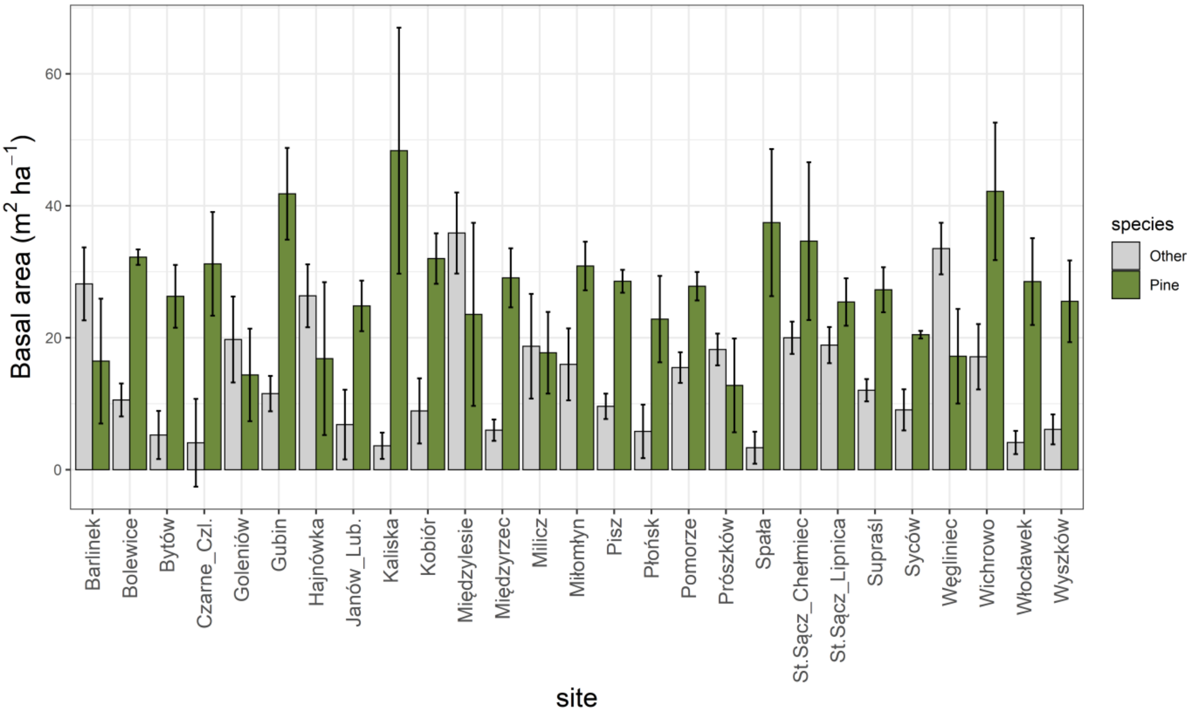

Fig. S2. Contribution of Scots pine and the associated species to the basal area of each sampled stand. Bars show mean values, and error bars show standard deviations.

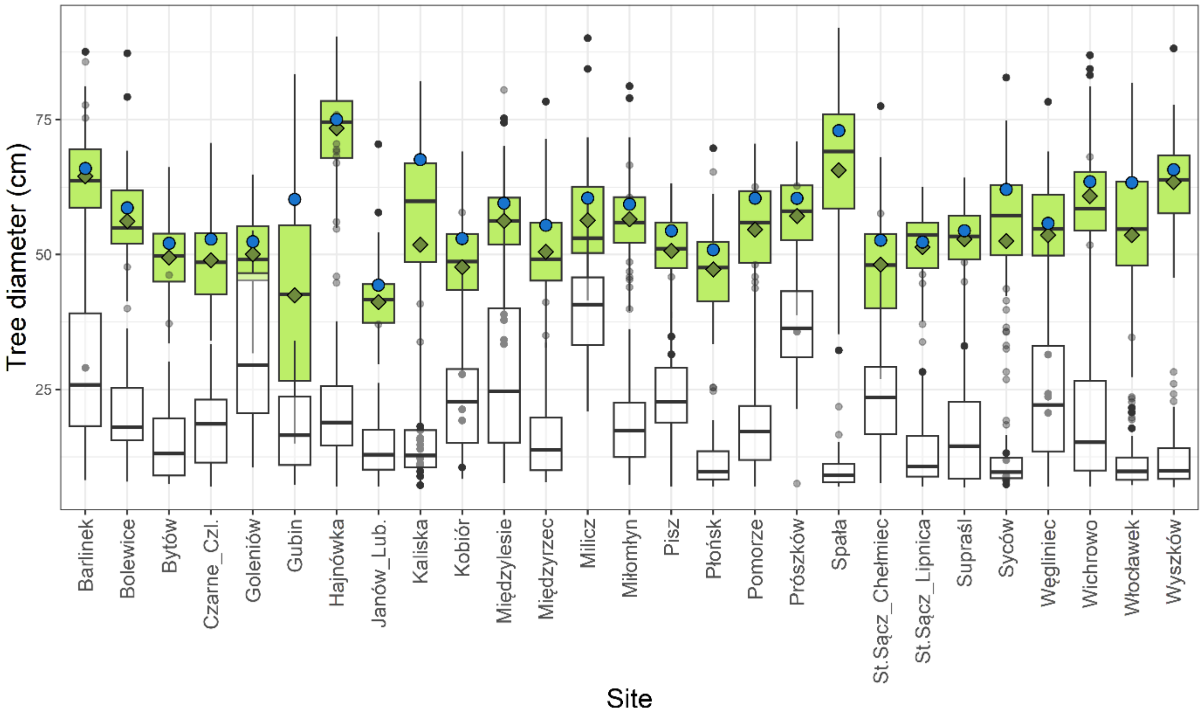

Fig. S3. A boxplot showing the variation of diameters of pine trees (filled boxes) and associated species (transparent boxes) in examined stands. A horizontal bar within the box indicates the median. Filled circles show the mean values for trees sampled for genetic analysis, and filled diamonds show the mean for all pine trees at each site.

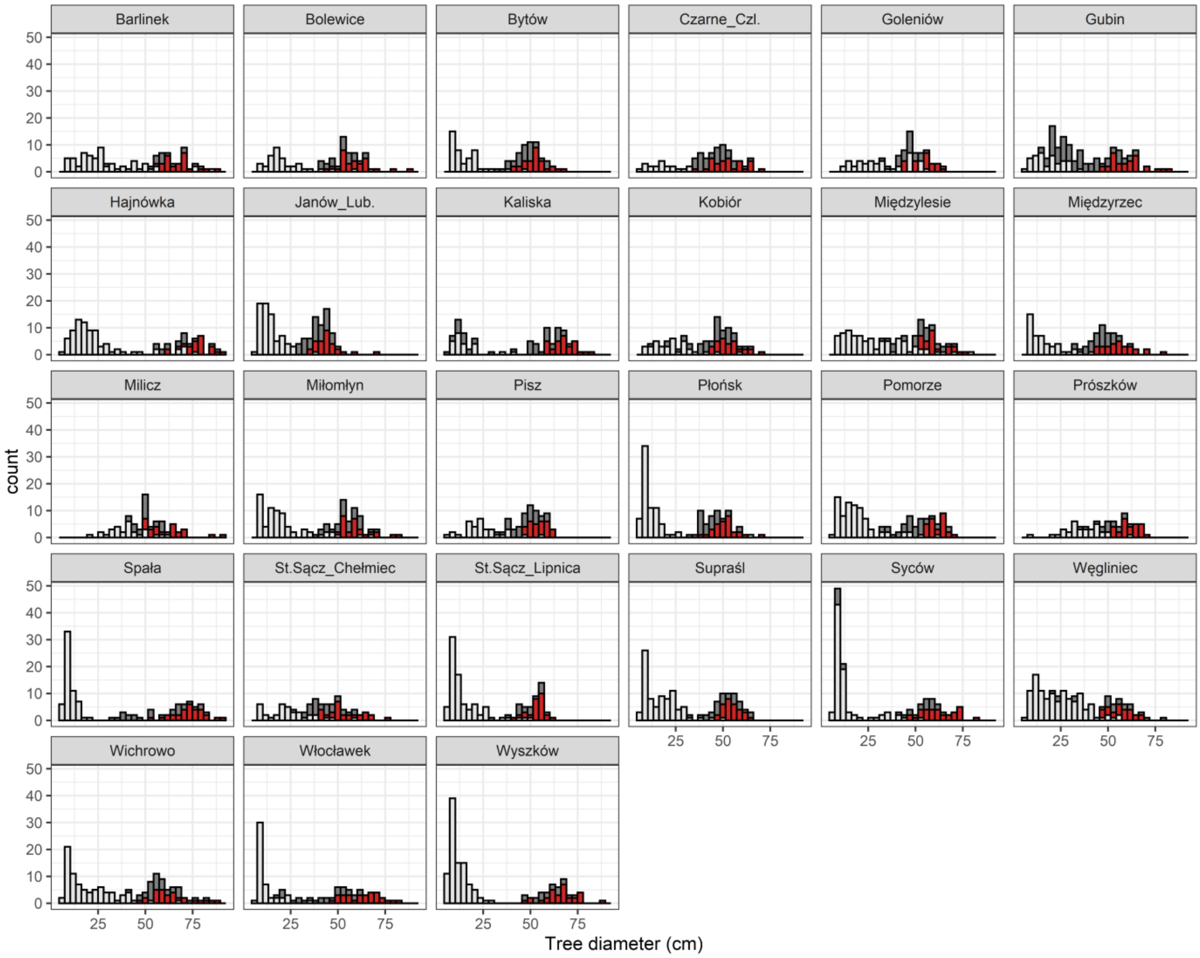

Fig. S4. A histogram showing the distribution of tree diameters in examined stands with Scots pine. Pine trees sampled for genetic analysis are shown in dark red, remaining pine trees in dark grey and all other species in light grey (in color).

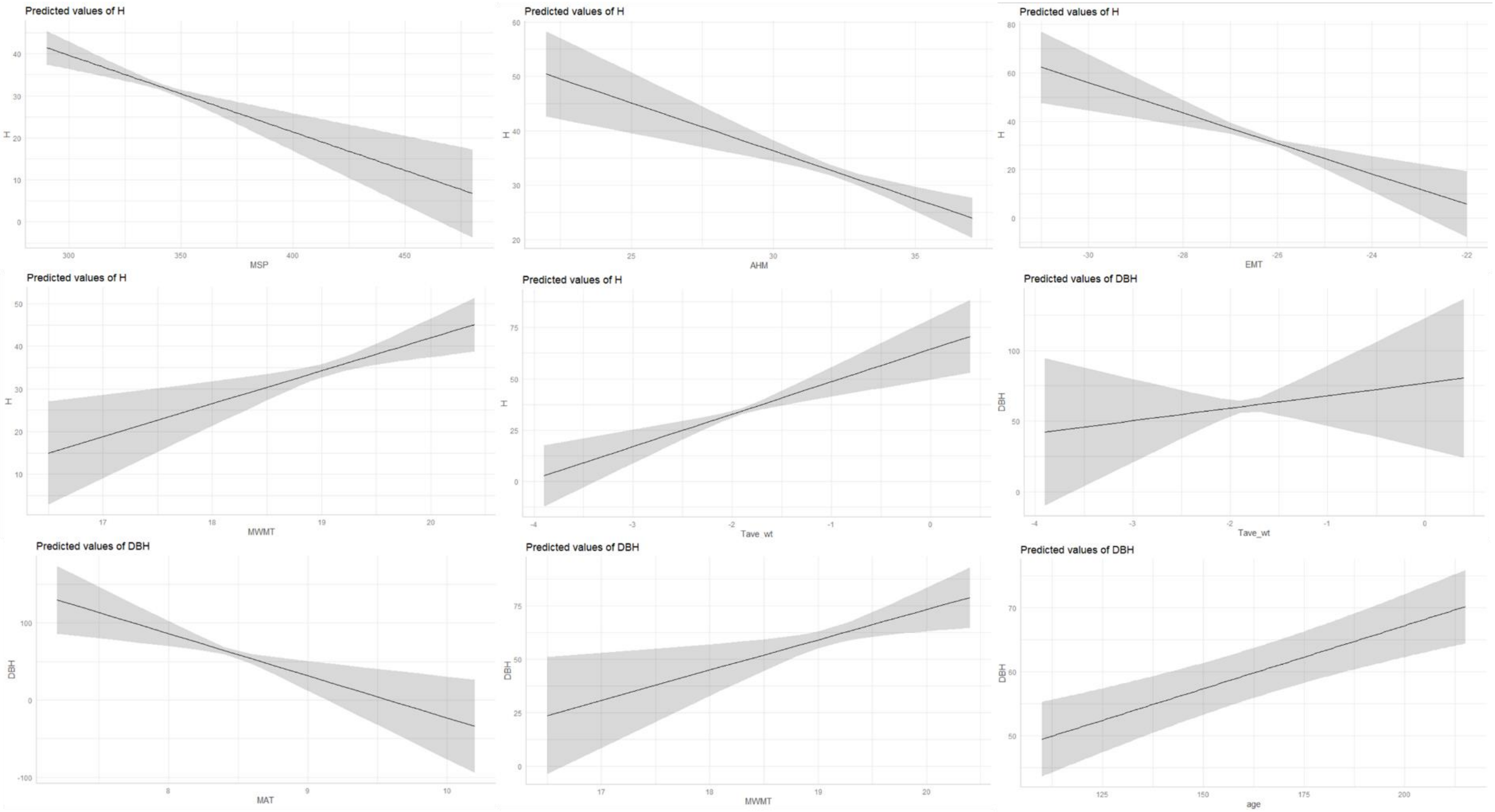

Fig. S5. Lmer models implemented in R v 4.2, for predicted significant climatic variables potentially influencing DBH and Height.

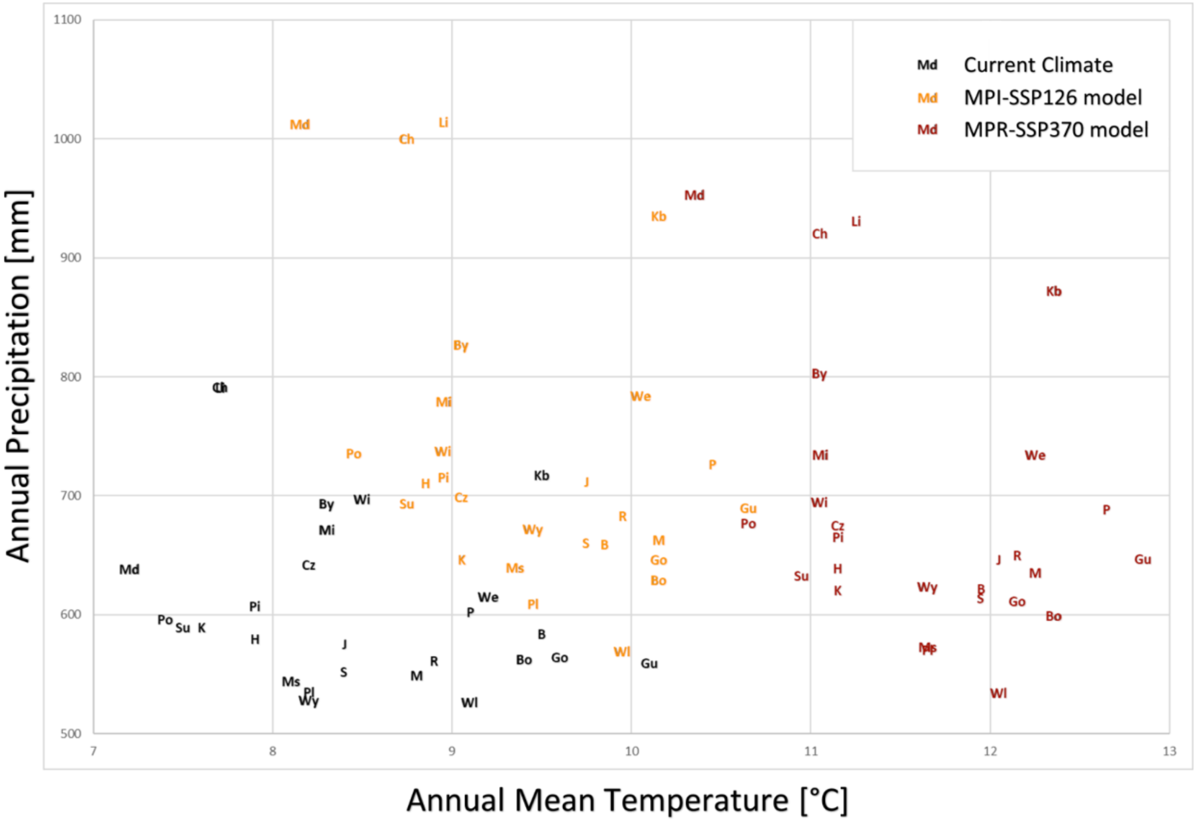

Fig S6.The Ecoplot visualizes future environmental conditions of the studied Polish populations, based on average temperature and average precipitation in the tested stands. The current conditions are indicated by black font, models of future conditions in orange (SSP-126) and brown (SSP-370).

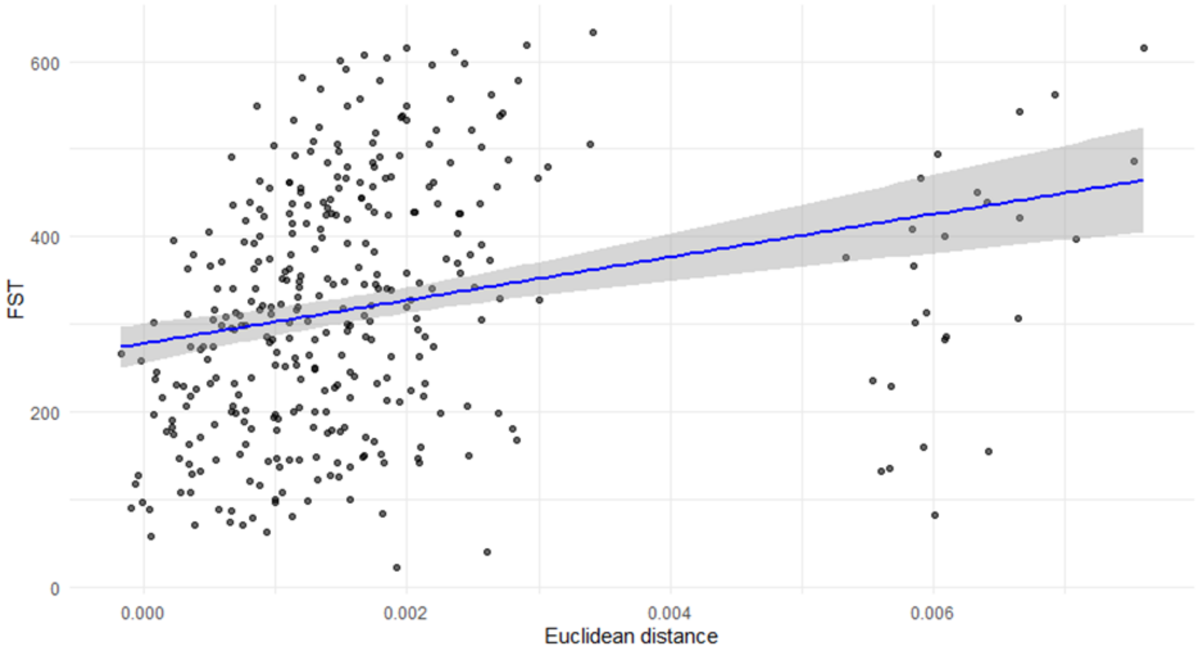

Fig. S7. Correlation between Euclidean distance and FST. Mantel statistic r: 0.254; significance: 1e-04

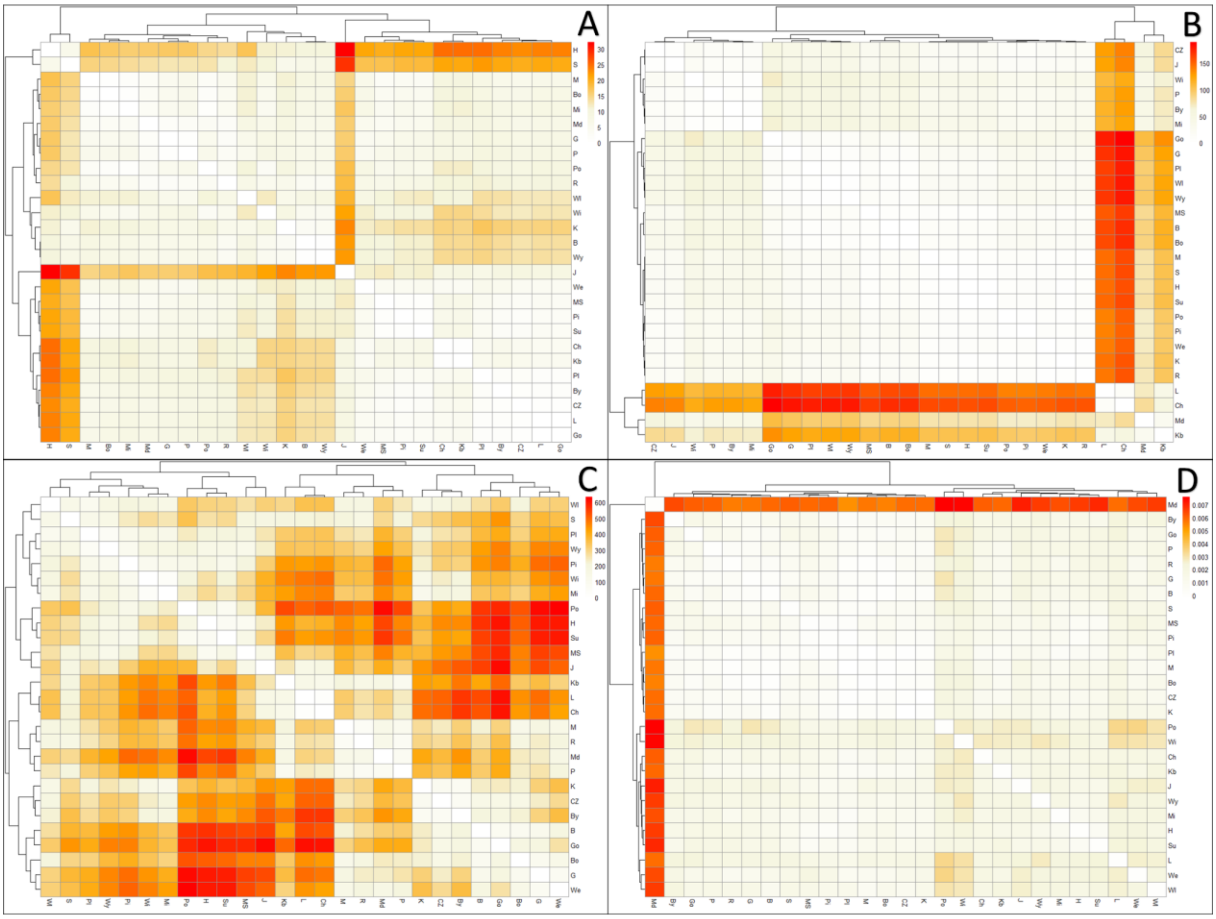

Fig.S8. Heatmaps with hierarchical clustering according to dissimilarity matrices. A - phenotypic dissimilarity; B - climatic dissimilarity (variables from linear mixed-effects model); C - Euclidean distance; D - genetic dissimilarity.

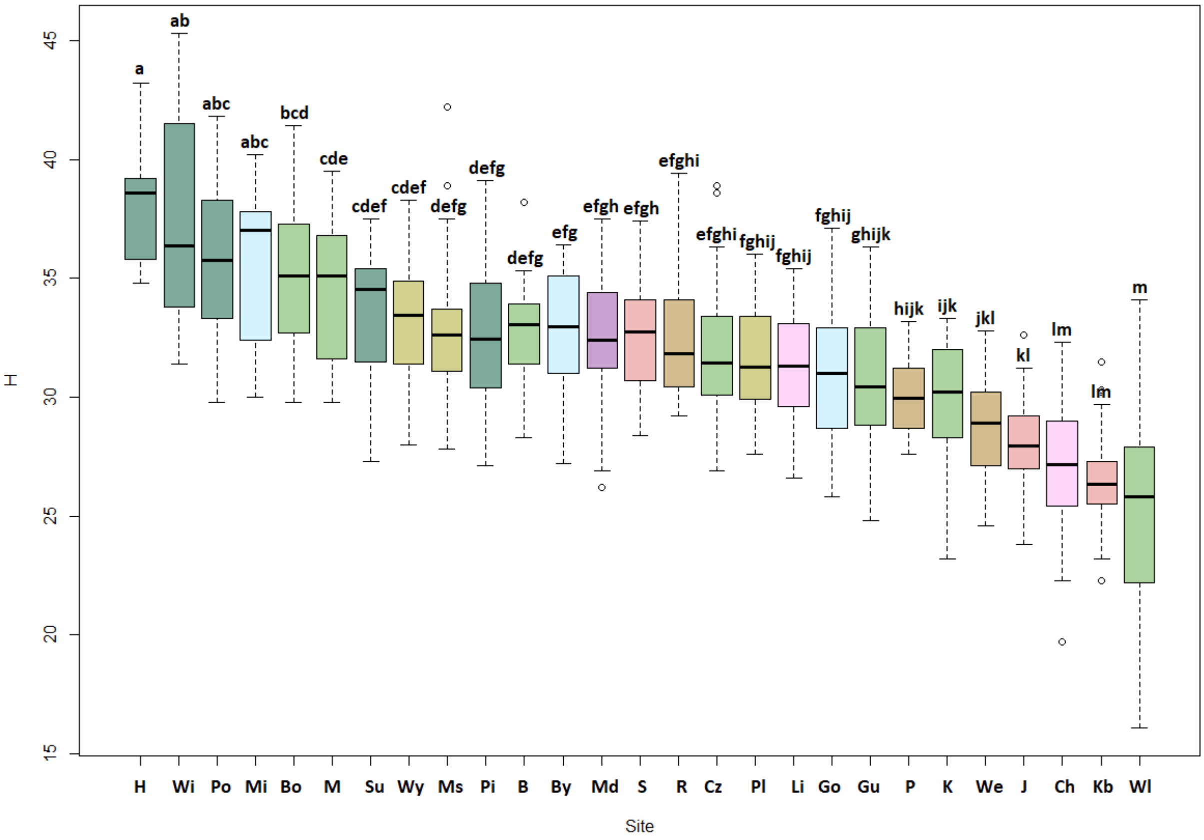

Fig. S9 – Height of individuals according to population and region. Colour of the bar indicates region, as in Figure S1. Letters above indicate group, according to the Tukey's HSD test calculated using ANOVA model.

Table S1. Silvicultural characteristics of sampled stands with Scots pine with detailed information about the associated species. DBH – diameter at 1.3 m above the ground, H – tree height, BA – basal area

Site	**Species**	**DBH (cm)**	**st.dev. DBH (cm)**	**H (m)**	**st.dev. H (m)**	**BA (m^2 ha^{-1})**	**st.dev. BA (m^2 ha^{-1})**
Barlinek	beech	29.4	17.2	24.8	6.1	28.2	5.5
Barlinek	pine	60.5	12.8	30.9	2.6	16.5	9.5
Barlinek	**mean**	34.1	20.0	25.7	6.1	22.3	9.5
Bolewice	beech	21.1	11.6	23.7	3.8	9.4	1.4
Bolewice	oak	23.1	8.6	21.3	3.5	4.7	2.4
Bolewice	pine	52.9	7.4	35.9	2.5	32.2	1.2
Bolewice	**mean**	33.7	18.0	27.9	7.2	17.1	13.2
Bytów	beech	15.9	5.3	16.2	4.8	1.3	1.6
Bytów	oak	41.7	6.4	24.7	2.8	2.8	0.8
Bytów	pine	46.8	6.6	32.6	2.3	26.3	4.8
Bytów	spruce	13.6	5.9	10.5	4.9	2.6	1.1
Bytów	**mean**.	28.2	17.2	20.8	10.9	9.0	11.6
Czarne_Czl.	beech	19.7	7.6	18.7	4.0	5.9	8.3
Czarne_Czl.	birch	9.2	2.1	10.8	1.2	0.4	
Czarne_Czl.	pine	45.7	7.7	30.9	2.9	31.2	7.9
Czarne_Czl.	**mean**.	36.0	15.4	26.2	7.3	19.6	16.0
Goleniów	beech	34.4	15.4	22.9	6.2	12.2	10.1
Goleniów	oak	30.0	10.9	22.4	4.0	7.1	3.8
Goleniów	pine	45.8	7.3	28.6	2.3	14.4	7.0
Goleniów	spruce	21.5	11.1	22.6	8.2	1.6	
Goleniów	**mean**	35.9	13.6	24.4	5.4	10.5	7.6
Gubin	beech	18.0	7.4	21.3	4.3	11.5	2.7
Gubin	pine	34.9	13.6	28.1	4.6	41.8	7.0
Gubin	**mean**	29.2	14.3	25.8	5.5	31.7	16.6
Hajnówka	oak	53.9	16.6	31.2	4.6	8.6	4.8
Hajnówka	hornbeam	17.5	6.1	19.3	4.0	8.2	3.4
Hajnówka	lime	28.7		24.8		1.3	

Site	**Species**	**DBH (cm)**	**st.dev. DBH (cm)**	**H (m)**	**st.dev. H (m)**	**BA (m^2 ha^{-1})**	**st.dev. BA (m^2 ha^{-1})**
Hajnówka	pine	68.1	12.2	36.9	1.4	16.8	11.6
Hajnówka	spruce	40.2	23.5	28.1	11.4	12.2	8.5
Hajnówka	**mean**	28.4	20.8	23.2	8.1	10.8	7.9
Janów_Lub.	beech	12.7	4.1	14.9	3.0	2.5	1.2
Janów_Lub.	birch	13.5	7.9	15.3	4.8	2.0	1.1
Janów_Lub.	oak	14.4	5.3	13.7	4.1	2.2	2.2
Janów_Lub.	fir	21.6		19.0		0.7	
Janów_Lub.	pine	38.9	5.5	26.7	1.6	24.8	3.8
Janów_Lub.	spruce	16.1	5.0	16.0	3.0	3.3	0.9
Janów_Lub.	**mean**	23.0	13.1	19.1	6.5	7.9	10.3
Kaliska	birch	15.7	8.2	15.3	4.6	3.6	1.9
Kaliska	pine	40.8	22.2	24.9	8.2	48.4	18.6
Kaliska	spruce	10.5		8.9		0.2	
Kaliska	**mean**	32.0	22.1	21.5	8.5	22.3	26.7
Kobiór	beech	30.3	15.4	20.7	7.1	5.3	4.4
Kobiór	birch	20.4	8.7	16.6	4.5	3.6	3.2
Kobiór	pine	43.7	11.5	25.4	4.0	32.0	3.8
Kobiór	spruce	20.9	5.4	16.0	4.8	5.1	
Kobiór	**mean**	34.1	15.2	21.7	6.2	13.6	14.0
Międzylesie	beech	35.4	14.4	25.4	7.1	11.3	5.3
Międzylesie	birch	31.4	8.6	26.9	3.0	2.4	1.6
Międzylesie	sycamore	33.2	11.7	24.8	3.4	4.5	4.5
Międzylesie	lime	22.2	10.6	19.6	6.6	5.1	3.4
Międzylesie	larch	80.5		33.9		10.2	
Międzylesie	pine	51.3	9.9	30.7	3.7	23.5	13.9
Międzylesie	spruce	27.0	16.7	20.5	10.0	20.1	11.1
Międzylesie	willow	23.7	3.1	19.2	3.5	0.9	0.2
Międzylesie	**mean**	33.3	17.6	23.5	8.9	11.9	11.5
Międzyrzec	birch	19.9	10.7	19.1	6.7	2.3	1.2

Site	**Species**	**DBH (cm)**	**st.dev. DBH (cm)**	**H (m)**	**st.dev. H (m)**	**BA (m^2 ha^{-1})**	**st.dev. BA (m^2 ha^{-1})**
Międzyrzec	oak	15.7	7.6	12.0	5.4	4.0	1.8
Międzyrzec	hornbeam	14.2	6.3	11.0	2.6	0.7	
Międzyrzec	rowan	7.8		10.3		0.1	
Międzyrzec	pine	46.2	6.7	31.1	2.1	29.1	4.5
Międzyrzec	**mean**	29.0	16.7	20.9	10.1	10.8	13.0
Milicz	beech	40.1	9.8	33.0	3.9	18.7	7.9
Milicz	pine	49.8	5.7	36.2	2.0	17.7	6.2
Milicz	**mean**	43.9	9.6	34.2	3.6	18.2	6.6
Miłomłyn	beech	25.9	16.5	23.1	6.0	6.3	6.0
Miłomłyn	oak	54.6	8.5	29.8	2.3	4.7	1.5
Miłomłyn	hornbeam	16.5	7.8	18.9	3.2	6.5	2.1
Miłomłyn	pine	53.5	7.5	35.1	3.8	30.9	3.7
Miłomłyn	spruce	38.1	9.4	28.0	4.1	4.8	0.7
Miłomłyn	**mean**	29.8	18.9	24.6	8.0	12.5	11.9
Pisz	pine	47.2	6.9	34.2	2.2	28.6	1.7
Pisz	spruce	24.9	10.2	22.6	7.2	9.6	1.9
Pisz	**mean**	35.7	14.2	28.2	7.9	19.1	10.3
Płońsk	birch	7.2		9.3		0.1	
Płońsk	black cherry	10.9	4.6	11.9	2.9	2.9	2.6
Płońsk	oak	14.7	15.3	11.3	6.2	2.2	3.0
Płońsk	hornbeam	25.4		17.7		1.0	
Płońsk	lime	7.8		6.7		0.1	
Płońsk	pine	43.6	6.3	30.5	2.5	22.8	6.5
Płońsk	spruce	12.6	3.2	10.8	2.8	2.3	0.8
Płońsk	**mean**	21.2	16.1	17.1	9.3	7.2	10.0
Pomorze	oak	9.6	0.1	10.3	0.8	0.1	0.0
Pomorze	pine	48.6	9.0	34.1	3.0	27.8	2.2
Pomorze	spruce	19.5	11.2	17.1	6.0	15.4	2.4
Pomorze	**mean**	27.0	16.8	21.5	9.3	17.3	10.9

Site	**Species**	**DBH (cm)**	**st.dev. DBH (cm)**	**H (m)**	**st.dev. H (m)**	**BA (m^2 ha^{-1})**	**st.dev. BA (m^2 ha^{-1})**
Prószków	oak	37.0	9.0	27.0	3.0	18.2	2.4
Prószków	pine	49.6	7.0	31.4	2.2	12.8	7.1
Prószków	spruce	7.6		6.2		0.1	
Prószków	**mean**	39.9	11.2	27.8	4.7	13.8	7.4
Spała	beech	18.2	5.2	11.9	2.0	1.1	
Spała	birch	7.5	0.4	9.2	1.1	0.2	0.1
Spała	black cherry	11.6	2.9	10.5	1.9	0.7	
Spała	oak	9.3	2.3	8.0	1.0	0.6	0.2
Spała	hornbeam	9.8	2.3	12.2	1.2	5.3	
Spała	fir	10.4	2.9	8.4	1.7	1.3	
Spała	rowan	7.3		6.6		0.1	
Spała	pine	57.4	15.5	30.9	2.8	37.4	11.1
Spała	**mean**	24.9	23.9	17.0	9.8	12.3	18.4
St.Sącz_Chełmiec	beech	24.2	12.5	21.5	5.7	15.0	5.8
St.Sącz_Chełmiec	fir	22.9	10.7	20.0	6.8	4.9	3.3
St.Sącz_Chełmiec	pine	42.1	8.4	27.2	1.9	34.6	12.0
St.Sącz_Chełmiec	**mean**	31.1	13.9	23.6	5.6	18.2	14.8
St.Sącz_Lipnica	beech	8.7	1.4	12.6	2.0	0.4	
St.Sącz_Lipnica	fir	14.1	7.0	13.9	4.3	12.0	1.3
St.Sącz_Lipnica	ash	9.3	1.9	14.7	2.0	1.7	
St.Sącz_Lipnica	sycamore	39.2	16.6	24.1	4.7	2.6	2.0
St.Sącz_Lipnica	larch	45.5	1.2	35.0	0.3	6.5	
St.Sącz_Lipnica	pine	49.3	8.1	30.4	3.9	25.4	3.6
St.Sącz_Lipnica	**mean**	19.4	15.1	16.9	7.4	9.8	9.9
Supraśl	beech	20.0	7.4	16.2	1.9	2.1	
Supraśl	birch	22.3	6.8	24.6	5.5	1.7	1.0
Supraśl	oak	19.8	5.8	18.7	4.7	4.2	1.6
Supraśl	maple	32.1	5.4	24.9	1.3	5.0	
Supraśl	hazel	8.3	0.9			1.1	0.6

Site	**Species**	**DBH (cm)**	**st.dev. DBH (cm)**	**H (m)**	**st.dev. H (m)**	**BA (m^2 ha^{-1})**	**st.dev. BA (m^2 ha^{-1})**
Supraśl	pine	51.1	8.0	33.8	3.1	27.3	3.4
Supraśl	spruce	18.4	10.6	16.5	7.0	4.7	3.3
Supraśl	**mean**	25.1	17.0	22.9	8.9	8.7	10.5
Syców	beech	14.6	12.4	13.6	2.3	5.1	4.1
Syców	birch	9.8	2.4	13.9	1.6	1.1	1.0
Syców	black cherry	10.2	2.3	10.5	1.3	0.9	0.5
Syców	oak	25.3	16.0	19.5	8.4	3.1	5.1
Syców	hornbeam	11.5	6.9	12.2	1.7	1.9	
Syców	fir	11.0	3.0	11.7	3.3	0.8	
Syców	hazel	8.6	1.3	8.4	0.0	0.2	
Syców	larch	14.3	5.8	16.5	4.4	0.7	
Syców	pine	40.2	22.1	25.4	10.3	20.5	0.6
Syców	spruce	27.3	12.9	20.5	9.2	2.3	0.3
Syców	willow	8.7		9.1		0.1	
Syców	**mean**	19.8	17.7	16.2	8.2	5.1	7.5
Węgliniec	beech	21.3	11.1	24.0	6.7	19.2	7.3
Węgliniec	birch	24.3	6.6	28.1	4.4	1.0	0.5
Węgliniec	pine	49.5	17.6	29.9	2.1	17.2	7.2
Węgliniec	spruce	31.8	11.4	27.8	5.5	18.5	7.8
Węgliniec	**mean**	27.1	15.2	25.6	6.4	15.6	8.9
Wichrowo	beech	11.8	5.7	11.1	2.1	0.4	0.4
Wichrowo	birch	20.9	10.5	21.3	6.2	1.9	0.7
Wichrowo	oak	21.8	11.1	18.4	8.2	2.4	2.3
Wichrowo	hornbeam	15.3	8.8	17.3	5.6	1.1	1.2
Wichrowo	rowan	7.5		8.3		0.1	
Wichrowo	maple	40.4		27.2		2.6	
Wichrowo	lime	21.0	13.9	20.7	8.2	6.3	2.9
Wichrowo	pine	58.3	8.6	37.6	3.6	42.2	10.4
Wichrowo	spruce	20.6	13.3	18.1	9.4	8.9	7.0

Site	**Species**	**DBH (cm)**	**st.dev. DBH (cm)**	**H (m)**	**st.dev. H (m)**	**BA (m^2 ha^{-1})**	**st.dev. BA (m^2 ha^{-1})**
Wichrowo	willow	9.5	2.3	10.3	0.6	0.1	0.1
Wichrowo	**mean**	30.6	20.8	23.7	11.4	9.5	15.5
Włocławek	birch	14.7	8.3	13.0	5.0	2.1	1.9
Włocławek	black cherry	10.3	3.2	9.3	2.2	2.1	2.5
Włocławek	pine	42.8	13.9	24.5	3.7	28.5	6.6
Włocławek	**mean**	22.8	17.7	15.4	7.7	10.9	13.7
Wyszków	birch	13.7	4.5	17.9	3.0	2.6	2.6
Wyszków	oak	15.3	5.9	14.6	3.6	1.8	1.7
Wyszków	hornbeam	18.9	4.0	16.7	0.9	0.6	0.2
Wyszków	hazel	8.8	1.7			1.4	1.0
Wyszków	pine	59.6	7.5	32.3	2.1	25.5	6.2
Wyszków	**mean**	19.4	18.3	20.8	7.6	7.0	10.6

Table S2. Values of Pearson correlation coefficient between climatic variables and stand characteristics. Bolded values are significant at P≤0.05. For definitions of climatic variables see Table S1.

Variable	DBH.all	pine.DBH	BA.ha	Pine_BA
MAT	0.26	-0.04	-0.22	-0.10
MWMT	-0.10	-0.06	-0.35	0.35
MCMT	**0.42**	0.06	-0.01	-0.44
TD	**-0.42**	-0.09	-0.20	0.58
MAP	-0.02	-0.23	**0.49**	-0.08
MSP	-0.03	-0.22	**0.43**	-0.11
AHM	0.07	0.18	**-0.50**	0.05
SHM	-0.03	0.15	**-0.47**	0.20
DD<0	-0.45	-0.08	0.02	**0.42**
DD>5	0.05	-0.09	-0.35	0.20
DD<18	-0.29	0.04	0.21	0.14
DD>18	0.07	-0.07	**-0.39**	0.15
NFFD	0.29	-0.05	-0.13	-0.10
bFFP	-0.03	0.15	0.22	-0.36
eFFP	0.28	-0.09	-0.06	0.00
FFP	0.17	-0.12	-0.14	0.18
PAS	-0.29	-0.08	0.21	0.30
EMT	0.37	0.04	-0.08	-0.30
Eref	0.10	-0.03	-0.16	-0.26
CMD	0.09	0.21	**-0.52**	0.04
DBH.all		0.20	0.13	-0.37
pine.DBH			0.12	**-0.40**
BA.ha				-0.23

DBH.all – mean diameter of all measured trees per site; pine.DBH - mean diameter of measured pine trees per site; BA.ha – basal area including all tree species per site ($m^2 ha^{-1}$); Pine_BA – proportion of pine in stand BA (see Table 2). **Table S1.** The list of climatic variables used in the analysis. The data were derived from ClimateEU v. 4.63 (Marchi et al., 2020) for a period 1991-2020.

Table S3. The list of climatic variables used in the analysis. The data were derived from ClimateEU v. 4.63 (Marchi et al., 2020) for a period 1991-2020.

Abbreviation	**Explanation**
MAT	mean annual temperature (°C),
MWMT	mean warmest month temperature (°C),
MCMT	mean coldest month temperature (°C),
TD	temperature difference between MWMT and MCMT, or continentality (°C),
MAP	mean annual precipitation (mm),
MSP	mean summer (May to Sept.) precipitation (mm),
AHM	annual heat : moisture index (MAT+10)/(MAP/1000))
SHM	summer heat : moisture index ((MWMT)/(MSP/1000))
DD<0	degree-days below 0°C, chilling degree-days
DD>5	degree-days above 5°C, growing degree-days
DD<18	degree-days below 18°C, cooling degree-days
DD>18	degree-days above 18°C, heating degree-days
NFFD	the number of frost-free days
bFFP	the Julian date on which FFP begins
eFFP	the Julian date on which FFP ends
FFP	frost-free period
PAS	precipitation as snow (mm) between August in previous year and July in current year
EMNT	extreme minimum temperature over 30 years (°C)
Eref	Hargreaves reference evaporation (mm)
CMD	Hargreaves climatic moisture deficit (mm)

Table S4. Loading matrix showing the contribution of stand characteristics and climatic variables into the first two Principal Components

Variable	PC1	PC2
DBH.all	-0.386	0.160
pine.DBH	-0.278	-0.019
BA.ha	-0.234	**-0.374**
Pine_BA	**0.555**	0.025
MAT	-0.103	**0.613**
TD	**0.509**	-0.091
MAP	-0.034	**-0.393**
bFFP	-0.286	**-0.443**
Eref	-0.241	0.313

DBH.all – mean diameter of all measured trees per site; pine.DBH - mean diameter of measured pine trees per site; BA.ha – basal area including all tree species per site (m^2 ha^{-1}); Pine_BA – proportion of pine in stand BA (see Table 2).

Table S5 Silvicultural characteristics of sampled Scots pine stands (DBH – diameter at 1.3 m above the ground, H – tree height, BA – basal area).

Site	DBH (cm)	st.dev.DBH (cm)	H (m)	st.dev. H (m)	Density (N ha^{-1})	st. dev. Density (N ha^{-1})	BA (m^2 ha^{-1})	st.dev.BA (m^2 ha^{-1})	Pine density (N ha^{-1})	Pine BA (m^2 ha^{-1})	Pine BA proportion
Barlinek	34.1	20.0	25.7	6.1	365	164	44.6	10.8	55	16.5	0.37
Bolewice	33.7	18.0	27.9	7.2	375	151	42.8	2.7	144	32.2	0.75
Bytów	28.2	17.2	20.8	10.9	370	66	31.5	3.8	150	26.3	0.83
Czarne_Czl.	36.0	15.4	26.2	7.3	285	137	34.3	12.3	185	31.2	0.91
Goleniów	35.9	13.6	24.4	5.4	295	66	34.1	8.7	85	14.4	0.42
Gubin	29.2	14.3	25.8	5.5	575	110	47.6	6.3	380	41.8	0.88
Hajnówka	28.4	20.8	23.2	8.1	445	145	43.2	12.5	45	16.8	0.39
Janów_Lub.	23.0	13.1	19.1	6.5	575	153	31.7	2.2	205	24.8	0.78
Kaliska	32.0	22.1	21.5	8.5	440	171	52.0	20.5	287	48.4	0.93
Kobiór	34.1	15.2	21.7	6.2	375	34	40.9	2.4	200	32.0	0.78
Międzylesie	33.3	17.6	23.5	8.9	535	157	59.4	14.7	110	23.5	0.40
Międzyrzec	29.0	16.7	20.9	10.1	400	99	35.1	3.9	170	29.1	0.83
Milicz	43.9	9.6	34.2	3.6	230	50	36.4	6.9	90	17.7	0.49
Miłomłyn	29.8	18.9	24.6	8.0	480	59	46.8	4.9	135	30.9	0.66
Pisz	35.7	14.2	28.2	7.9	330	62	38.2	3.5	160	28.6	0.75
Płońsk	21.2	16.1	17.1	9.3	515	232	28.6	7.3	150	22.8	0.80
Pomorze	27.0	16.8	21.5	9.3	545	90	43.3	4.4	145	27.8	0.64
Prószków	39.9	11.2	27.8	4.7	230	38	31.0	7.1	65	12.8	0.41
Spała	24.9	23.9	17.0	9.8	430	297	40.0	11.6	135	37.4	0.94
St.Sącz_Chełmiec	31.1	13.9	23.6	5.6	600	85	54.6	9.5	240	34.6	0.63
St.Sącz_Lipnica	19.4	15.1	16.9	7.4	940	113	44.3	0.9	130	25.4	0.57
Supraśl	25.1	17.0	22.9	8.9	545	97	39.3	4.0	130	27.3	0.69
Syców	19.8	17.7	16.2	8.2	535	263	29.5	3.2	125	20.5	0.69
Węgliniec	27.1	15.2	25.6	6.4	670	232	50.7	7.8	80	17.2	0.34
Wichrowo	30.6	20.8	23.7	11.4	555	122	59.3	7.0	155	42.2	0.71
Włocławek	22.8	17.7	15.4	7.7	500	159	32.6	5.9	180	28.5	0.87
Wyszków	19.4	18.3	20.8	7.6	570	213	31.6	7.3	90	25.5	0.81