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RESEARCH

Effective population size of adult and offspring cohorts as a genetic monitoring tool in two stand-forming and wind-pollinated tree species: *Fagus sylvatica* L. and *Picea abies* (L.) Karst.

Heike Liesebach[1] · Pascal Eusemann[1] · Aki M. Höltken[2] · Ute Tröber[3] · Oleksandra Kuchma[4] · Manuel Karopka[5] · Frank Becker[6] · Ralf Kätzel[6] · Barbara Fussi[7]

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Abstract

Genetic diversity is considered to be a prerequisite for adaptation and adaptability as it is a key element of biological diversity. However, the monitoring of genetic diversity has tended to be ignored in biodiversity monitoring. We report a comprehensive genetic monitoring effort in two dominant forest tree species, which was started with a baseline survey in 12 European beech populations and 10 Norway spruce populations in Germany. The standardized experimental design is based on collecting samples of at least 250 adult trees, and 400 natural regeneration and 400 seed samples and their genotyping with 15–16 high-resolution SSR markers. In addition to commonly used mean values across the markers to quantify genetic diversity, we placed special emphasis on various marker-based, pedigree-based and demographic models for estimating the contemporary effective population size N_e of the different generations. In both beech and spruce, no variation in genetic diversity with mean values across markers was detectable between the studied stands and between age cohorts. We detected that stable allelic diversity in progeny generations is ensured by sufficient gene flow from surrounding forests. However, estimates of effective population size show marked differentiation among populations and among age cohorts. Natural regeneration samples appear to converge on the parent generation, while seed samples show a clear bottleneck effect. The N_e parameter can be used to derive conclusions for sustainable natural regeneration management in forest stands and for seed stand approvals including adequate seed collections for appropriate artificial regenerations.The sibship frequency-based method for N_e estimates is presented as much more robust than the widely used LD estimates, which often fail for samples with too weak relatedness. Despite the distinct kinship structure in our monitoring plots, the contemporary effective population size proves to be an essential parameter for assessing the integrity of the reproductive system.

Keywords Forest genetic monitoring · Effective population size · Ratio N_e/N · Effective number of parents · Parentage analysis · Allelic diversity · SSR markers · Natural regeneration · Forest Reproductive Material (FRM)

✉ Heike Liesebach
heike.liesebach@thuenen.de

[1] Thuenen Institute of Forest Genetics, Federal Research Institute for Rural Areas, Forestry and Fisheries, Sieker Landstrasse 2, 22927 Grosshansdorf, Germany

[2] Department of Forest Genetic Resources, NW-FVA, Prof.-Oelkers-Strasse 6, 34346 Hann. Muenden, Germany

[3] Unit for Forest Genetics and Tree Breeding, Public Enterprise Sachsenforst (SBS), Bonnewitzer Strasse 34, 01796 Pirna, Germany

[4] ISOGEN GmbH & Co. KG, Buesgenweg 2, 37077 Goettingen, Germany

[5] Department Forest Nature Conservation, Forest Research Institute Baden-Wuerttemberg, Wonnhaldestrasse 4, 79100 Freiburg, Germany

[6] Department of Forest Ecology and Monitoring, State Forestry Institute Brandenburg, Alfred-Moeller-Strasse 1, 16225 Eberswalde, Germany

[7] Bavarian Office for Forest Genetics, Forstamtsplatz 1, 83317 Teisendorf, Germany

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Introduction

Genetic diversity is a key element of biological diversity and is responsible for variability within species. It is therefore considered a prerequisite for adaptation and adaptive capacity, but has been neglected in biodiversity monitoring (Pearman et al. 2024). The ongoing evolutionary process with several components, as recombination through sexual reproduction, gene flow, selection, random drift and mutation, affects the spatial and temporal structures of genetic diversity within species. Moreover, for certain species, these processes are also very selectively influenced by human activities such as livestock and crops. Even though our trees in managed forests are still largely wild species today, evolutionary forces can be out of balance. Silvicultural strategies may act in different ways (reviewed by Namkoong et al. 2002), and this is not only by changing environmental conditions, but also, e.g., by (selective) reduction of population sizes or by fragmentation, which enhances random drift. Further, forest management practices may strongly intervene in the reproduction process, especially in case of artificial stand establishment including the transfer of forest reproductive material, up to intense breeding activities in clonal forestry.

The idea of genetic monitoring in forest tree populations aims to analyze reproductive processes of species and the transmission of genetic diversity from one generation to the next and to better understand these processes. Knowledge in this field will help to develop species-specific measures to preserve forest genetic resources and to evaluate the sustainability of forest management systems. This is explicitly not about the protection of species threatened with extinction, for which genetically underpinned species protection concepts are needed outside of forestry. Initial concepts for genetic monitoring in forest tree populations have been created and later evolved by Namkoong et al. (1996, 2002). In Germany, several pilot studies or smaller funded projects on genetic monitoring have been conducted so far (Degen et al. 2008; Maurer et al. 2008; Steiner et al. 2010; Kätzel et al. 2011; Konnert et al. 2011). At European or transnational scale, some more recent studies using different marker types, sampling designs and parameters were performed (LIFEGENMON: Fussi et al. 2016; FORGER: Kramer et al. 2016).

The main objective of genetic monitoring, the analysis of temporal trends in genetic variation within populations and species (e. g. Hvilsom et al. 2022), would involve the analysis of multiple complete life cycles, which is virtually impossible for long-lived species such as trees. However, a limited number of generations can be studied with sampling of multiple age classes per population. Subsequently, besides other assessments, population genetic parameters are determined based on the number and frequency of genetic variants (alleles and genotypes), usually averaged over markers. Parameters such as allele numbers, effective allele numbers, allelic richness, observed and expected heterozygosity, or fixation index are commonly used to quantify and compare genetic diversity between experimental units.

Moreover, the effective population size (N_e) has already been proposed as a useful parameter for genetic monitoring in general, and in forest trees particularly. It is defined as the size of an ideal population in which mating is random, sex ratios are equal, generations are discrete, and variation in reproductive success is random (Waples 2005). In an ideal population, census size N and effective size N_e are the same. Originally this parameter has been set aside for the time being as too difficult to achieve in forest tree populations (Namkoong et al. 2002). In the meantime, this parameter is mentioned as the first in an enumeration of various genetic parameters to be collected for genetic monitoring purposes in genetic conservation units of forest trees in Europe (Aravanopoulos et al. 2015). However, due to the many different methods and models, it is difficult to obtain reliable and comparable data. Basic demographic models that account for sex ratios, fluctuating population sizes, or variances in reproductive success were developed by Wright (1938). Later on, they were extended to become more applicable to different life history situations, e. g. for continuous populations with a neighborhood structure (Wright 1946). Increasingly, models for estimation of effective population size using genetic marker information are being used (reviewed in Waples 2022). Currently, the usefulness of N_e and N_e/N ratios in forest tree populations is doubted on the one hand (Fady and Bozzano 2021; Santos-del-Blanco et al. 2022), and emphasized as indicators of genetic diversity conservation in the context of global Convention on Biological Diversity activities (Hoban et al. 2020, 2021) on the other hand.

In practice, it is still very difficult to develop experimental designs, to collect appropriate data, and to apply appropriate genetic diversity assessment methods to provide meaningful conclusions for practice, as many idealizing assumptions for the application of certain formulas can hardly be met. Among others, nonrandom samples with an excess of relatives are specifically mentioned by Waples (2016) as a need for more systematic assessment. Here we attempt to make a practical contribution focusing on two central European tree species, *Fagus sylvatica* L. (European beech) and *Picea abies* (L.) Karst. (Norway spruce), which are commonly stand-forming and dominant species with large populations. The focus here is not on species protection, but on forest management and in particular on forest regeneration. These two wind-pollinated model species, a deciduous tree and a conifer, were selected for a new stage forest genetic monitoring in Germany because of their high ecological and

economic importance. A joint research project with national funding was initiated to collect data for a baseline survey, including the sampling of three cohorts per population—adult trees, natural regeneration and seeds—and genotyping them with established microsatellite marker sets. The data sets were completed by a number of stand characteristics and phenotypical traits. In this comprehensive study using a standardized experimental design, we try to identify common and generalizable patterns in reproductive behavior and genetic diversity parameters in different age cohorts, as well as possible deviations from them.

The paper presented here focuses on the following aspects:

- What generalizable patterns in mating system characteristics and in the transmission of genetic diversity from the adult generation to its offspring can be derived for the two model species, European beech and Norway spruce?
- What conclusions can be drawn for practical forest management considering preservation of genetic resources, natural regeneration, recommendations for seed stand approvals, and seed harvest?
- Can we derive conclusions for future experimental designs, required sample sizes and suitable parameters to assess and evaluate genetic diversity in forest trees (with a special emphasis on N_e estimates)?

Material and methods

Experimental design and studied populations

In total, 22 monitoring plots are included in this study, 12 plots for beech and 10 plots for spruce, located within the species natural range and well distributed in Germany (Table 1, map in Online Resource 1). The average age of beech stands is about 140 years, and that of spruce stands is 110 years. Monitoring plots were established as described in the national "Guidance on the implementation of genetic monitoring for stand-forming tree species" (BLAG 2008; Konnert et al. 2011) with an extensive plot of 200m x 200m and a nested intensive plot of 100m x 100m. According to the described sampling scheme, all potentially reproductive trees in the intensive plots (minimum 15 cm DBH) were sampled. If this resulted in fewer than 250 trees due to lower density, additional samples were taken in the extensive sections. The natural regeneration cohort consisted of a representative sample of 200 seedlings within the intensive section and four natural regeneration clusters of 50 individuals each. These seedlings were about 0.5–2.0 m tall and are assumed to be from one to about three regeneration events. Seeds were collected from 20 spatially well distributed adult trees of all diameter classes in the intensive sections, and embryos were prepared from 20 seeds per parent tree. The number of 20 mothers for seed harvesting corresponds to the number specified in the regulations for the FRM in Germany for common tree species. Applying this scheme, three cohorts with minimum 250 adults, 400 natural regeneration samples and 400 seeds per monitoring plot were collected. Only a few deviations from this experimental

Table 1 Overview on 22 monitoring plots with their sample sizes in three cohorts: adult trees (N_c = Census number), natural regeneration and seeds

Beech					Spruce				
Pop	State	Adults (N_c)	Nat. Reg.	Seeds	Pop	State	Adults (N_c)	Nat. Reg.	Seeds
BB1	Brandenburg	274	400	400	BB3	Brandenburg	251	400	248
BB2	Brandenburg	250	399	399	BW2	Baden-Wuerttemberg	252	404	400
BW1	Baden-Wuerttemberg	250	396	400	BW3	Baden-Wuerttemberg	250	402	400
BY1	Bavaria	331	400	403	BY3	Bavaria	425	400	400
BY2	Bavaria	295	400	401	HE2	Hesse	251	200	414
HE1	Hesse	250	400	958	NI2	Lower Saxony	250	318	366
MV1	Mecklenburg-Pomerania	250	400	399	RP3	Rhineland-Palatine	250	400	400
NI1	Lower Saxony	249	200	898	SN3	Saxony	339	400	400
SN1	Saxony	257	400	400	ST2	Saxony-Anhalt	250	200	283
SN2	Saxony	546	400	400	TH2	Thuringia	327	395	-
ST1	Saxony-Anhalt	284	401	458					
TH1	Thuringia	358	395	400					
	Total	3594	4591	5916		Total	2845	3519	3311

design occurred due to different local availabilities. A total of 14,101 beech samples and 9,675 spruce samples were genetically analyzed (Table 1).

This design might violate some of the usual assumptions for calculating population genetic parameters. First, our samples are not random samples of larger populations because we focused on local subpopulations. Therefore, the samples cover a limited area as part of that larger population, and they necessarily contain an increasing proportion of kinship in the three age cohorts from adult trees to natural regeneration and to seed samples. This is not necessarily a disadvantage, as we analyze immobile tree populations with a natural neighborhood structure, and it is part of what we want to capture. In addition, partially overlapping generations must be accounted for. Seed samples are from a one-year reproductive event as they have been harvested directly from each mother tree, and generation overlap is impossible here. The same holds for natural regeneration, representing a few reproductive events of the same adult population. The sampled adult populations may definitely consist of one generation (in the case of artificial stand establishment, as found in many spruce stands) or of possibly partially overlapping generations in the case of silvicultural management including natural regeneration (in many of the beech populations studied). Currently, many of the studied stands are protected as nature reserves or forest genetic resources. However, the documented or estimated age of the observed adult generation indicates that they were established as part of usual silvicultural management practices at the time. In the case of previous natural regeneration, the current adult trees are likely the result of an initiated process with a rather limited number of reproductive events. Therefore, we assume that partially overlapping generations play only a minor role in our experimental design.

Genotyping

For beech as well as for spruce, we used nuclear microsatellites (SSRs), since well-established marker sets with 16 and 15 markers were available. We consider the markers to be unlinked and selectively neutral. Thus, a marker type was used that has already proven itself in many other population genetic studies (Stefanini et al. 2022). Beech markers based on developments from Lefèvre et al. (2012) were optimized for Beckman Coulter capillary sequencers by Eusemann et al. (2017). Markers for spruce were summarized from several sources (Pfeiffer et al. 1997; Hodgetts et al. 2001; Scotti et al. 2002; Besnard et al. 2003; Rungis et al. 2004; Fluch et al. 2011) and optimized at the beginning of the joint project (Technical details regarding DNA extraction, PCR amplification and fragment analysis are provided in Online Resource 1). Due to the large sample size, DNA extraction and genotyping was done in four independent laboratories. Ring tests with a collection of reference samples for the two species were carried out between four involved labs to detect possible allele shifts and to ensure a reliable and consistent allele scoring.

Data analysis

Base diversity parameters per population and per cohort were calculated with Genalex Version 6.503 (Peakall and Smouse 2012) as mean values with their standard errors across the loci analyzed: Mean number of alleles per locus (A/L), effective number of alleles (A_e), observed heterozygosity (H_{obs}), and expected heterozygosity (H_{exp}). The allelic richness approach was used to correct for differences in sample sizes among populations and the three cohorts. The minimum sample sizes amounted to 200 for beech as well as for spruce cohorts (see Table 1). Rarefaction was done with simple random sampling and 100 replicates (SAS Version 9.4: proc surveyselect, proc allele, proc means). Tests for significant differences between mean diversity values of age cohorts were performed using SAS proc glm. The tests for possible deviations from Hardy–Weinberg equilibrium were performed with SAS using proc allele with 10,000 permutations to approximate exact p-values.

Parentage analyses were performed with COLONY, a programme for inferring parentage and sibships from multilocus genotype data, version 2.0.6.6 (Jones and Wang 2010; Wang 2013). This package can handle null alleles as well as typing errors and furthermore reconstruct unsampled parents. The following parameter settings were commonly used in all analysis: diploid and monoecious species with polygamy for males and females, inbreeding allowed, unknown population allele frequency, Full-Likelihood model (FL), Number of runs = 1, medium run, and high precision. For runs with the adult generation alone, we used the parameters no sibship prior and not updating allele frequency, whereas for parentage analysis including offspring samples and candidate parents we used the updating of allele frequencies. More specifically, for parentage analysis of seed samples we used their known maternal sibships together with medium sibship prior, assumed male family size = 3, female family size = 20 (or = 50 for HE1 and NI1), probability that a father is included in the male candidates = 0.5, and probability that a mother is included in the female candidates = 1. For parentage analysis of sampled natural regeneration we used a medium sibship prior, male and female family size = 3, probability that a father is included in the male candidates = 0.5, and probability that a mother is included in the female candidates = 0.9. All runs were replicated with at least three different random seeds to check the reproducibility. The estimated number of parents (sampled and unsampled) N_p contributing at least to one offspring is relatively constant over replicated runs (variation coefficient ~ 2–3%). A bias with an overestimated number of

parents, as mentioned by Sefc and Koblmüller (2009), is not to be feared here, as our sample sizes and marker numbers are considerably larger than their simulated datasets with no more than 11 loci and sample sizes of 25 and 80 offspring individuals, respectively.

Estimates of effective population size were made using various models. To get contemporary N_e estimates on our local subpopulations here, we used the sibship frequency (SF) based N_e estimator for nonrandom and random mating and their 95% confidence limits provided in the COLONY outputs for single samples of adult populations as well as for parent–offspring data. Wang (2016) compared several methods based on SF model, linkage disequilibrium (LD), heterozygote excess or molecular coancestry using extensive simulations on single sample estimators under idealized conditions. He showed that the method based on SF model is more accurate and robust than other methods. An evaluation of the realized sample sizes for the reliability of N_e estimates was performed with replicated COLONY runs with random subsets of samples (see Online Resource 8).

For comparison purposes, we have likewise used the widely accepted N_e based on the LD model, which is implemented in the software NeEstimator version 2.1 (Do et al. 2014). Furthermore, we applied a demographic N_e based on the variances of reproductive success using gamete numbers from parentage analysis under the precondition of a constant adult population size: $N_e = 4*N_p / (2 + Var_k)$, where N_p is the number of contributing adults (sampled and unsampled parents) and Var_k is the variance of their relative gamete contributions. In addition, we propose to calculate the parameter called "effective number of parents N_{ep} " by assuming that each effective parent contributes the same number of gametes: $N_{ep} = 1 / \sum p_i^2$, where p_i are the relative frequencies of contributions to offspring from i parents (calculation analogous to the effective number of alleles from allele frequencies). The numbers of contributed gametes per sampled and unsampled parent individual for N_{ep} and the demographic N_e were taken from the COLONY parentage analysis.

The N_e/N_c ratio was calculated for the three cohorts of each monitoring plot using the SF-based N_e (non-random mating) and the census size N_c of adult trees sampled and genotyped for each plot. A critical discussion of the correctly linked N_e and N values is given by Waples (2005) and Palstra and Fraser (2012). Because we are attempting to use contemporary estimates for local subpopulations, we hypothesize that the number of sampled adult trees from plots in our experimental design should be representative for closed forests of both species in the observed age classes.

Results

Base diversity parameters

The commonly used diversity parameters as mean number of alleles per locus (A/L), the effective number of alleles (A_e), and the level of observed (H_{obs}) and expected heterozygosity (H_{exp}) do not show any significant differences, neither between the 12 or 10 populations of each species nor between the sampled cohorts of adults, natural regeneration and seeds. Only allelic richness is somewhat lower in seeds than in the adults (Online Resources 2 and 3). As a representative example, the effective number of alleles A_e per population and per age cohort is given in Fig. 1. Five spruce and two beech loci have extensive null allele frequencies leading to highly significant deviations from HWE (estimated null allele frequencies up to 0.12 … 0.24). Apart from these cases, the vast majority of tests (85% in beech and 72% in spruce) show no significant deviations from HWE (p-values in Online Resource 4). Additional loci with null alleles at lower frequencies could be at least partially responsible for these remaining deviations from HWE. Together with the very low selfing rates (see Table 3), we can assume that the matings in our sample plots are not far from random mating.

Allele loss and its compensation

The nearly consistent allelic diversity of the three age cohorts of each population (parameters A/L, A_e, A_r Fig. 1, Online Resource 3) should not hide the fact that there is considerable exchange in allelic composition. Many alleles present in the adult trees were not passed on to the sampled offspring, and many alleles found in the natural regeneration or seed samples obviously originated from unsampled trees. Each age cohort contains about 87% of the total alleles (range 77–94%) sampled in the respective population with only slight differences between beech and spruce (Table 2, single population data in Online Resource 5).

The loss of alleles from adults to the offspring generation becomes understandable when one considers that only about 30% of the old trees in our experimental design contributed to the analyzed offspring. However, a mean proportion of 43% resp. 44% of natural regeneration plants in the beech and spruce monitoring plots have only one sampled parent, and 36% resp. 43% of sampled natural regeneration has no parents among the sampled adult trees (Summarized results in Table 3). The unassigned offspring of 1.5% in beech and 4% in spruce seed samples could be explained by mistakes in seed handling or genotyping errors.

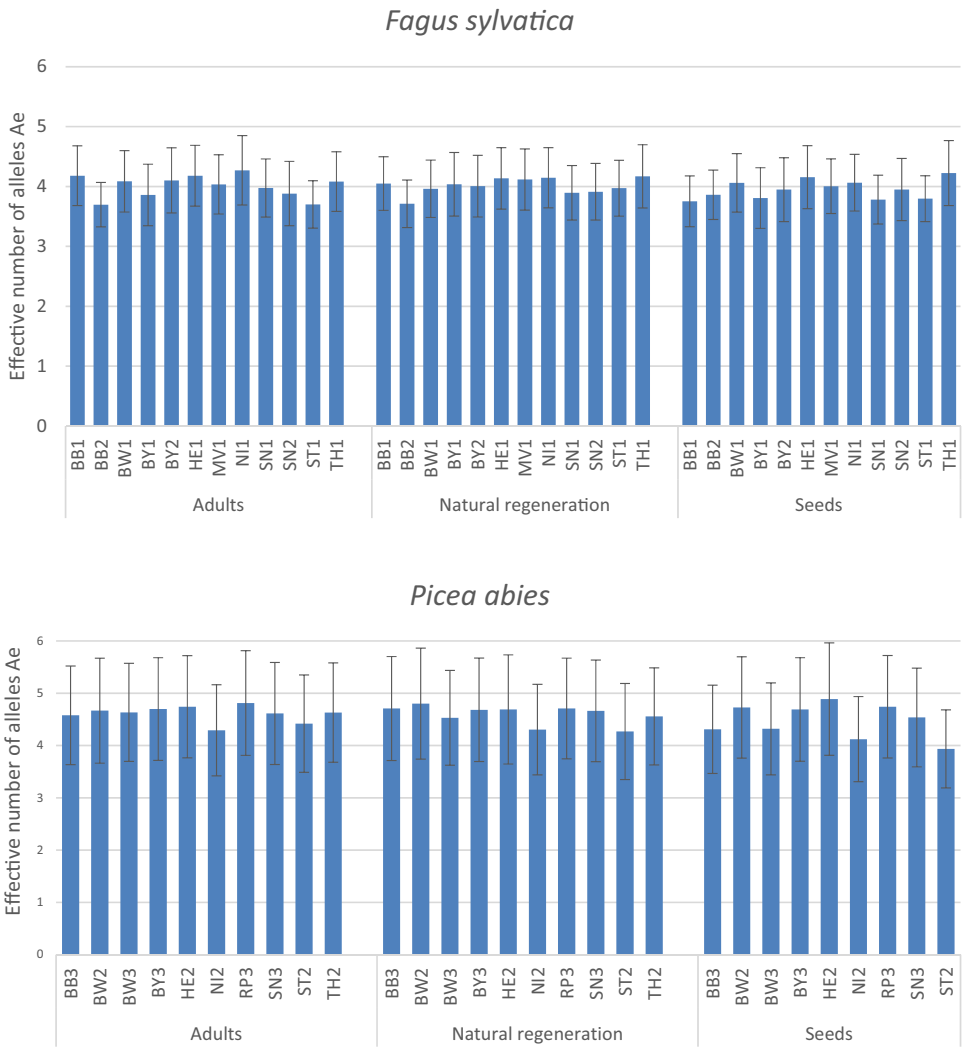

Fig. 1 Effective number of alleles A_e of 12 beech (Panel A) and 10 spruce (Panel B) monitoring plots and three age cohorts each (for population abbreviations see Table 1) calculated as mean values and standard errors across the analyzed loci (for other diversity parameters as Mean number of alleles per locus, Allelic richness, Mean observed and expected heterozygosity see Online Resources 2 and 3)

Table 2 Percentages of alleles present in different cohorts (number of all detected alleles over all marker loci per population = 100%), means and range over the populations

		Alleles in adults	Alleles in natural regeneration	Alleles in seeds
Beech	Mean	87%	88%	86%
	Range	81–92%	77–93%	80–91%
Spruce	Mean	87%	87%	84%
	Range	82–92%	80–94%	79–89%

The estimated number of unsampled parents contributing to the offspring was derived from COLONY pedigree reconstruction and is considerably larger than the number of sampled contributing trees (Table 3). We can observe this complete balance in allele numbers between the age cohorts due to intense gene flow, as the sampling plots are located within extensive forest areas dominating by beech or spruce.

Effective population size

The effective population sizes show remarkable differences among the monitoring plots and especially among the three age cohorts. Figure 2 illustrates the N_e estimates derived from sibship frequencies under non-random mating conditions. This picture contrasts strongly with the mean values of the diversity parameters described before, which show practically no differentiation among populations and among cohorts.These contemporary N_e estimates for the samples of adult populations are related to the size of their founder populations (Waples 2005), whereas the N_e values of the offspring samples (natural regeneration and seeds) are related to the participating sampled and unsampled parents (current adult trees).

Eleven of twelve adult beech populations descended from relatively homogeneous number of founders averaging 158, with one exceptional case of BB1 (Fig. 2). This beech population BB1 with $N_e = 37$, was the only one managed in a large-scale shelterwood cutting (Eusemann et al.

Table 3 Summarized results of parentage analysis for natural regeneration and seed samples from 12 beech and 10 spruce populations: mean values and standard errors across populations (for single population results see Online Resource 6)

	Beech (12 populations)		Spruce (10 populations)	
	Natural regen.	Seeds	Natural regen.	Seeds
% Outcrossed offspring with 2 sampled parents	20.3 ± 3.6	46.1 ± 3.7	11.9 ± 3.1	31.6 ± 4.2
% Outcrossed offspring with 1 sampled parent	43.1 ± 3.8	51.7 ± 3.5	44.4 ± 2.0	63.7 ± 3.5
% Selfed offspring	0.10 ± 0.06	0.75 ± 0.19	0.03 ± 0.02	0.72 ± 0.19
% Unassigned offspring (unsampled parents only)	36.5 ± 6.6	1.5 ± 0.4	43.7 ± 4.3	4.0 ± 1.0
N_p (Number of contributing parents)	229 ± 11	198 ± 18	245 ± 17	174 ± 10
N_p Sampled	87 ± 8	89 ± 6	88 ± 7	68 ± 5
N_p Unsampled	142 ± 11	110 ± 16	157 ± 12	107 ± 9
Sampled adults without offspring	213 ± 21	211 ± 21	197 ± 15	212 ± 17

2021). A very restricted number of seed-trees produced the current mature generation in population BB1, in contrast to predominantly small-scale natural regeneration using larger numbers of seed-trees in the other beech populations. Adult spruce stands, however, show much more fluctuations compared to beech, furthermore extremely high N_e values occur in some cases. This is mainly due to artificial stand establishment with partially mixed seed, while only BB3, NI2 and ST2 originate from natural regeneration (Eusemann et al., in preparation). Marker-related limitations in spruce such as many rare and frequent null alleles cannot be the cause here, since the phenomenon does not occur in seed samples.

Samples from natural regeneration also show strongly fluctuating N_e values. Here, the spatial distribution of the targeted representative sampling of 200 saplings, the more or less close family structures and higher percentages of correlated paternity in the four sampled clusters, and number of reproductive events between the monitoring plots may have an effect. The average values for natural regeneration samples across populations appear to be slightly lower than for adult populations (Table 4), but without significance. However, a drastic and highly significant reduction in effective population size is recorded for the seed samples. For both species, the mean N_e of seed samples amounts to about 60 with relatively low fluctuations (Fig. 2, Table 4). This result is not surprising since all of these samples consist of equally sized half-sib seed samples from 20 individual mother trees per monitoring plot, and therefore representing very similar sibship structures.

In addition to the two N_e measures derived from sibship frequencies for non-random and random mating, we calculated different N_e estimates using several independent models (Fig. 3, Table 4, Online Resource 7). As a third marker-based N_e estimate, we employed the LD method, resulting in approximately similar values to the other estimates at minimum allele frequencies of 0.05 and 0.02, respectively. Fourth, we determined a demographic N_e, and fifth, we calculated the effective number of parents N_{ep}, whereby the last two estimates are derived directly from relative gametic contributions from parentage analysis.

Comparison of these independently determined values shows relatively good agreement for all populations and age cohorts of beech and for all seed samples of spruce populations (see Fig. 3). With the exception of the N_e from LD model, they always have the consistent order $N_{ep} < N_e$-SF (non-random) $< N_e$-SF (random) $< N_e$-demographic and are highly correlated ($r \sim 0.99$). N_e estimates from the LD model vary somewhat more and are sometimes above or below demographic N_e estimates. However, N_e values from the LD model fail in 7 of 10 adult spruce populations and in some of their natural regeneration samples, as the estimates clearly exceed the theoretical maximum of the double

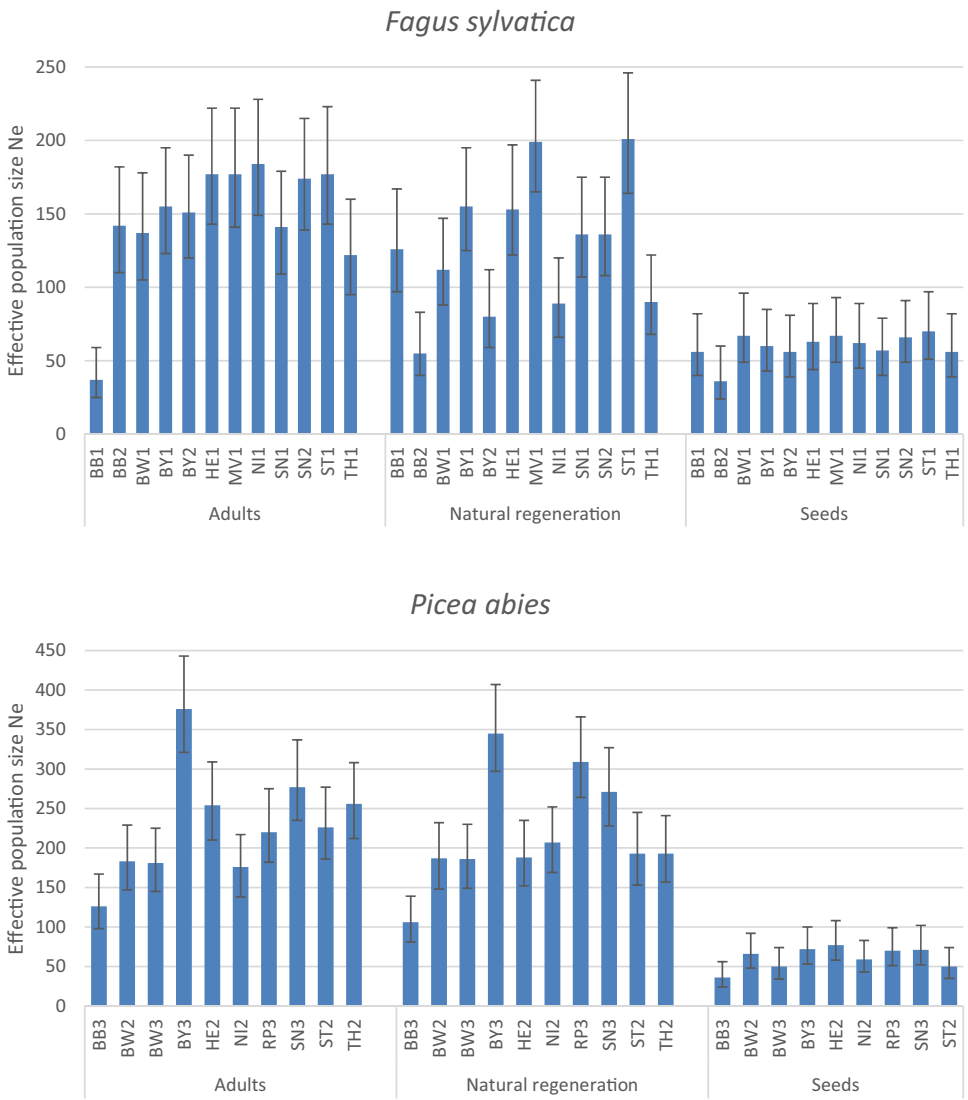

Fig. 2 Effective population sizes based on the Sibship Frequency model (SF, nonrandom mating) and their 95% confidence limits in beech (Panel A) and spruce (Panel B) monitoring plots with three age cohorts each

sample size and mostly show infinite upper confidence limits (Online Resource 7).

Besides the different N_e estimates, the ratio N_e/N_c was calculated from the SF based N_e under non-random mating of each cohort and the census number N_c of sampled adult trees (Table 4, single population data in Online Resource 7). The age cohorts of adults and natural regeneration samples show comparable mean N_e/N_c ratios for beech with ~ 0.5, and for spruce with ~ 0.8, whereas the seed samples of both species have lower ratios of about 0.2.

Recommendations for experimental sample sizes usually refer to true N_e values. Therefore, an assessment of the reliability of the data can only be attempted in retrospect. In our study, the number of genotyped individuals per population and cohort was always larger than the calculated N_e values from the SF model (non-random mating) and thus in line with the recommendations of Wang (2016), with a single exception for the adult spruce stand HE2 with N = 251 and $N_e = 254$ (Online Resource 7). A more detailed analysis was performed for some case studies with random subsamples of different sizes to check for reaching a plateau in N_e values according to England et al. (2006) (Online Resource 8).

Discussion

The question of how to measure and evaluate genetic diversity is an important topic in the discussion on biological conservation. In particular, such knowledge is urgently needed for forest trees, as forests are key ecosystems and habitats for many species worldwide. Recently, the extent to which general recommendations should be applied to forest tree populations has been controversely discussed (Fady and Bozzano 2021; Hoban et al. 2021; Santos-del-Blanco et al. 2022). Our work should contribute some practical aspects in this context considering local populations *of Fagus sylvatica*

Table 4 Summarized effective population sizes for 12 beech and 10 spruce population plots (three age cohorts each), and ratio N_e/N_c, calculated from the SF based N_e under non-random mating of each cohort and the number of sampled adult trees (for N_c see Table 1), given as mean values and standard errors across the populations (for single population values see Online Resource 7)

		Absolute number of parents (sampled + assumed unsampled)	N_e (SF model, non-random mating)	N_e (Demographic, variance in reproductive success)	N_{ep} (Effective number of parents)	Ratio N_e/N_c
Beech	Adults	0 + 175	147.8 ± 11.6	184.2 ± 14.9	118.2 ± 8.4	0.518 ± 0.052
	Nat. reg	87 + 142	127.7 ± 13.1	148.4 ± 15.9	110.8 ± 10.2	0.448 ± 0.055
	Seeds	89 + 110	59.7 ± 2.6	68.3 ± 2.9	57.8 ± 2.4	0.209 ± 0.015
Spruce	Adults	0 + 205	227.5 ± 21.9	282.0 ± 25.6	166.8 ± 14.2	0.794 ± 0.044
	Nat. reg	88 + 157	218.5 ± 22.1	257.4 ± 26.0	167.8 ± 15.1	0.770 ± 0.065
	Seeds	68 + 107	61.2 ± 4.5	71.6 ± 5.5	59.3 ± 4.2	0.223 ± 0.018

and *Picea abies*, two dominating, stand-forming and wind-pollinated tree species. These tree species are not considered endangered in Central Europe, but are often subject to forest management that strongly interferes with the reproductive process in particular. They are under pressure for several reasons: as a result of anthropogenic development, their populations are often reduced, fragmented and composed of gene pools influenced by artificial seed transfer and forest management. They are also increasingly stressed by the effects of climate change, such as drought, heat and secondary biotic factors. The evaluation of reproductive processes is therefore of particular interest.

What characteristics should we expect in an intact reproductive system of a local population of a common stand forming and wind-pollinated tree species? As a kind of benchmark against which to assess the genetic impact of silvicultural measures or changing environmental conditions, we assume that:

1. Demographic characteristics: the census population size (N_c = number of mature trees per area) is not changing over the generations, the population is connected via gene flow with surrounding forests, sexual reproduction occurs as a predominantly outbreeding process with high variance in individual reproductive success, a type III survivorship curve (Szabó 1931; Petit and Hampe 2006), repeated sexual reproduction events occur during a longer reproductively mature stage, including possible overlapping generations
2. Population genetic characteristics: stable genetic diversity over the generations, not only for parameters as mean values across the analyzed markers, but explicitly as well as for the effective population size N_e.

However, these assumptions do not mean that we expect so-called ideal populations with $N_e = N_c$. On the one hand, variance in reproductive success for our two model species is much larger than expected in a Poisson distribution, including a remarkably high proportion of non-breeding members of the adult populations. On the other hand, several reproductive events contribute to the next generation, and overlapping generations cannot be excluded. Additionally, we have larger continuous distribution patterns combined with a restricted seed and pollen flow. These factors lead us to expect an effective population size significantly smaller than the census size in intact reproductive systems of local populations. Perhaps, the theoretical expectations of a ratio $N_e/N \sim 0.5$ from Nunney (1993) are a good reflection of the given situation in our two study species.

Generalization of experimental data on the transmission of genetic diversity through sexual reproduction

The uniform experimental design of our study with a set of plots for each of two tree species allows to draw some general conclusions about reproductive processes and the transmission of genetic diversity to the next generation. The mean values across the markers of the commonly used population genetic parameters A/L, A_e, H_o, H_e or allelic richness derived from adequate sample sizes do not differ between plots or between age cohorts. The initial findings suggest that there are stable genetic structures in our plots and their progeny. High levels of gene flow by pollen (51% beech and 63% spruce) and by seeds (36% beech and 43% spruce) can balance these parameters, as only 30% of adult trees in the plots contributed to the sampled progenies. Consequently, the relevant parental generation is not restricted to the defined plots.

The analyzed adult populations on average have the largest effective population sizes compared to the other age cohorts. With one exception (Eusemann et al. 2021), N_e values of adult beech populations are quite similar. In spruce populations, N_e has larger fluctuation ranges, which can be explained by artificial establishment in many stands with partially mixed seed sources. In the natural regeneration cohorts, N_e is somewhat lower on average with larger fluctuations. We can expect an ongoing process in which the

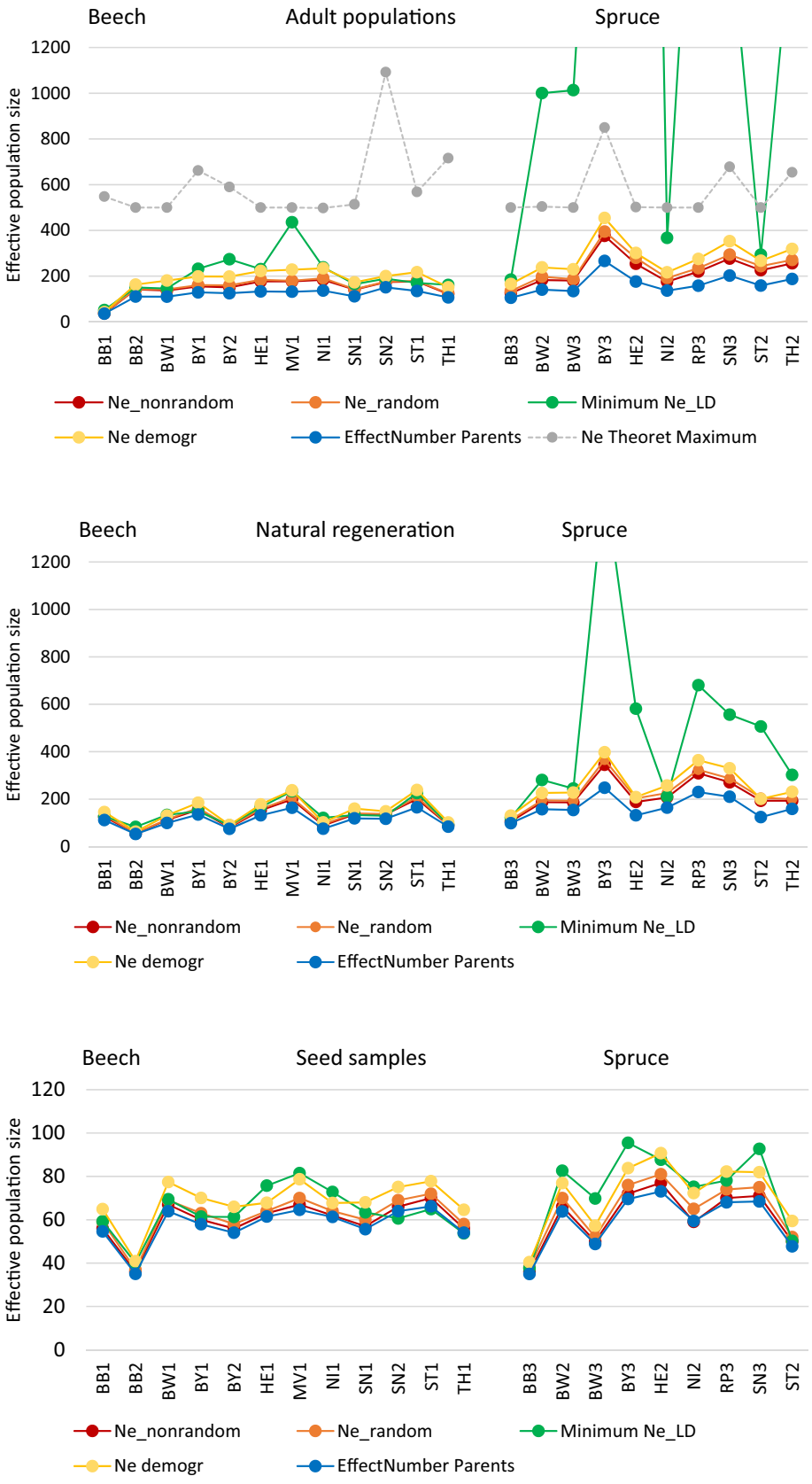

Fig. 3 Comparison of five different N_e estimators for samples of 12 beech and 10 spruce monitoring plots. The lines connecting the dots help to visualize the ranking of the N_e values of the different models. Panel A: Adult populations, Panel B: Natural regeneration, Panel C: Seed samples. For adult populations, the theoretical maximum of twice the sample size is also included (see also Trask et al. 2017). This would mean that there is no variance in reproductive success in the demographic model, or that there is no relatedness between the individuals in the sample, as they are each descended from two unrelated parents

number of reproductive events increases and the effective population size approaches that of the adult cohorts.

The mean N_e/N_c ratio of about 0.5 in adult beech populations and their natural regeneration samples is nearly the same as the theoretical expectation for populations with repeated reproduction events (Nunney 1993). Therefore, we conclude a largely intact reproduction system for that species. The mean N_e/N_c ratio for spruce, with predominantly artificial stand establishment and partially used seed mixtures, is actually even greater than the theoretical expectation.

Although the allelic diversity of seed cohorts is comparable to adults and natural regeneration, their effective population sizes are significantly lower. This reflects their descendance from only 20 mothers in one year: the alleles occur in more similar combinations due to the half-sibling structure, which narrows the genetic base and their evolutionary potential in case of using such seeds for artificial reforestation.

Many other studies in forest tree populations use diversity parameters as average values across the markers combined with estimates of contemporary effective population sizes. Predominantly balanced levels of genetic diversity between parent and natural regenerations were observed, for example, for *Fagus sylvatica* in 12 German populations with $N_e = 88…700$ in adults ($N = 100…200$) and $N_e = 138…657$ in seedlings ($N = 144…266$) using the LD model (Müller et al. 2018). For a *Quercus petraea* population, the estimates are $N_e = 164$ ($N = 246$) for adults and $N_e = 166$ for seedlings ($N = 487$) using the SF model (Eusemann and Liesebach 2021). Relatively stable mean values and somewhat more fluctuating N_e values due to small sample sizes were observed for *Quercus robur*, *Betula pendula*, *Populus tremula*, *Alnus glutinosa* and *Fraxinus excelsior* in a study by Verbylaitė et al. (2023). A reduced genetic diversity in the offspring compared to the adult generation due to small population size and/or isolation was observed e. g. in artificial Douglas fir stands by Neophytou et al. (2019) using average values and by Wojacki et al. (2019) using averaged parameters and additional N_e estimates. In other studies, a reduced genetic diversity becomes visible only by considering N_e estimates. In a Norway spruce study, allelic richness was comparable between seed samples from natural stands, seed stands and seed orchards. On the contrary, N_e was strongly reduced in seed from seed orchards due to the limited number of clones ($N_e = 25…31$) compared to seed from seed stands ($N_e = 88…108$) usingg the LD model (Sønstebø et al. 2018). Very similar results were published by Ruņģis et al. (2019) for Norway spruce with comparable levels of diversity derived from average values, but considerable reduced N_e estimates in seed from seed orchards ($N_e = 48…68$) compared to an adult population ($N_e = 251$) and its regeneration ($N_e = 167$). A discordant ranking of average value diversity parameters and N_e estimates was also observed by Liesebach et al. (2014, 2015) for *Fagus sylvatica* progeny samples and by Degen et al. (2015) for *Fagus sylvatica* and *Quercus robur*.

Conclusions for forest practice

The results of our study underline the importance of large reproductive units in stand-forming and wind-pollinated tree species, appropriate to their reproductive biology, for the *maintainance of genetic diversity* for future forest generations. For example, the pan-European minimum requirements for dynamic conservation units of forest tree genetic diversity (Koskela et al. 2013) recommend minimum sizes for conservation populations of at least 500 reproducing trees for widespread and stand-forming tree species. The German concept for conservation and sustainable use of forest genetic resources also suggests areas of at least 20 ha for protected genetic conservation forests (Paul et al. 2010).

The presented data support the recommendation to apply silvicultural treatments with longer regeneration periods to cover multiple reproductive events and mast years, and to promote *small-scale natural regeneration* instead of, e.g. heavy shelterwood cutting, to ensure a representative transfer of genetic diversity to the next generation in managed forests. However, the example of adult population BB1, which underwent a severe bottleneck with a very limited number of seed parents, shows a possible compensation of deficiencies, as the genetic diversity including effective population size of the natural regeneration cohort and seed sample is comparable to that of the other populations. Such compensation can be successful already in the following generation, but only if the stand is not isolated and gene flow and migration occur.

Minimum requirements for the *approval of seed stands* for wind-pollinated and stand-forming tree species should consider the reproductive biology with their need for gene flow to maintain genetic diversity, and therefore, besides a number of quality criteria, should focus on large populations. Ideally, the same minimum criteria should be applied as for gene conservation units. In practice, a compromise must often be found between ideal conditions for sexual reproduction and local constraints and opportunities. *Commercial seed collections* in approved seed stands should aim for a genetic diversity of the parent population that is as representative as possible. Seed harvests from only 20 trees, as required by the current legal guidelines in Germany and often practiced to date, definitely lead to a bottleneck in the progeny. This bottleneck is much more pronounced in smaller and/or isolated populations, and is further exacerbated when the seed trees are spatially clustered. This narrow genetic base could prove critical if such seed is used

elsewhere to establish artificial populations. Seed collection of at least 30–40 seed trees, with spacing of 20 m (seed stands < 10 ha) to 50 m (seed stands > 10 ha), as recommended for *Quercus robur* and *Q. petraea* by Degen et al. 2012 (unpublished report), would be more appropriate. More detailed effects on the number and spatial distribution of seed trees will be derived from pollen and seed dispersal patterns (Eusemann et al., in preparation). A mixture of seed yields over several years could mimic longer reproductive periods and should be discussed, or even a mixture of seed sources or a phased reforestation should be considered.

Conclusion for suitable monitoring parameters

In general, experimental series with standardized designs allow generalizations and improve the confidence in conclusions compared to single case studies. Sample sizes of a few hundred individuals have been shown to reliably determine genetic diversity parameters of local populations of stand-forming and wind-pollinated tree species. Mean values over the analyzed loci estimates of variants (allele and genotype counts and frequency) and all derived parameters such as H_{obs}, H_{exp}, A_e are easily determined. These parameters can detect extreme bottlenecks or selfing, and rarefaction approaches can compensate for different sample sizes. However, they are not able to detect other processes, especially changes in the mating system. Another drawback is that comparability between studies may be difficult or impossible due to different marker sets. We are convinced that effective population size N_e, which is largely independent of the marker sets used, is an indispensable additional parameter in the assessment and comparison of genetic diversity. Especially for insights into demographic and evolutionary processes, it cannot be replaced by the usual averages over markers. However, it remains difficult to choose among the different theoretical N_e models to be used in genetic monitoring in order to best meet the conditions of sampling design, sample size, and marker sets. A number of problems related to large continuous tree populations and resulting limitations in N_e estimates are discussed in detail, e.g., by Santos-del-Blanco et al. (2022). However, it is worth going beyond the commonly used LD model by comparing the different N_e models using empirical data as in our study.

In principle, all used measures in our study, whether marker-, demographic-, or pedigree-based, provide comparable results under the precondition of sufficient relatedness. For all populations and age cohorts of beech and for all seed samples of spruce populations, they are in the same magnitude per population, highly correlated with each other and thus mutually supportive. In contrast, extremely high LD-based N_e values in some of the adult spruce stands can be taken as a fairly reliable indication of "jumping gene flow" caused by the transfer and mixing of seed sources. The closer the relatedness in a sample is, the more consistent the N_e estimates of the different models are. If the relatedness is too low, the LD model based on 2-locus combinations is at a disadvantage, as there is obviously no significant linkage disequilibrium for many of the 2-locus combinations. These are the cases where large populations become indistinguishable from infinite populations (Waples and Do 2010) and the upper confidence limits become infinite. This disadvantage seems to be overcome by the SF model, where all loci are used simultaneously instead of only two loci at a time. Waples and Do (2010) suggest that "LD and sibship one-sample methods might have roughly comparable levels of performance". However, they acknowledge that it "is very difficult to obtain reliable estimates for large populations" with the LD model. Our empirical data from some adult spruce stands show very high N_e estimates from the LD model, exceeding the theoretical maximum of twice the sample size, combined with partially infinite upper confidence limits. The idea of a theoretical maximum of N_e comes from the demographic model of the variance of reproductive success, but we have generalized it here for the in principle highly correlated and mutually supporting N_e models.

We recommend the SF method, which provides very robust estimates of N_e that have never exceeded the theoretical maximum and have finite confidence intervals even for samples with low relatedness. In studies with parentage analyses that provide gamete counts including those from unsampled parents, demographic N_e based on the variances of reproductive success and the effective number of parents N_{ep} work equally well. In the words of Neel et al. (2013), our sampling window is obviously smaller than the breeding window for the species under consideration. This sampling procedure in combination with distinct neighborhood structures perhaps makes it difficult to find a clear demarcation from other parameters such as neighborhood size (Wright 1946) or effective number of breeders (Waples 2005). In general, N_e estimates are optimal to monitor genetic diversity over longer time periods as technical developments lead to changing marker sets, e.g., microsatellites, smaller or larger sets of SNPs, or sequence data. In addition, N_e estimates allow comparison between different studies with different marker types when comparable study designs and sample sizes are used. Currently, none of the existing N_e models can fully represent the complexity of forest tree populations and do not require simplifying model assumptions. However, this should not prevent us from exploiting the power of contemporary N_e estimates, e.g. to improve forest management systems or to produce high-quality forest reproductive material.

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Data availability Upon acceptance for publication of the manuscript all SSR genotypes will be made available on OSF (Open Science Framework, https://osf.io, link: https://doi.org/10.17605/OSF.IO/WVZFB).

Declarations

Competing interests The authors have no conflicts of interest to declare.

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