

<https://doi.org/10.1007/s10592-024-01600-2>

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.

Conservation Genetics SPRINGER

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Online Resource 1: Material description (A) and technical details on genotyping (B)

A – Location of 22 plots for genetic monitoring in Germany



B – Technical details on genotyping

DNA was extracted using the DNeasy Plant Kit (Qiagen, Hilden), the InnuPure C16 Plant kit (Analytik Jena, Jena) following the manufacturer's protocols, and a modified CTAB extraction protocol (Dumolin et al. 1995).

Genotyping was done with 16 SSR loci for beech and 15 SSR loci for spruce. For both species, the markers were divided into two multiplex sets of seven and eight loci, respectively. The identity and sources of all loci, the dyes used and the observed fragment length ranges are listed in the following table.

Marker name	Reference	Abbreviation (Online Resources 4 and 5)	Dye for Beckman Coulter CEQ8800	Dye for ABI 3730xl	Fragment length (bp), this study
<i>Fagus sylvatica</i>					
Multiplex 1					
sfc_0036	Lefèvre et al. (2012)	SFC36	BMN-6	HEX	91-119
FS1-15	Pastorelli et al. (2003)	FS115	Cy5	ROX	82-146
sfc_1143	Lefèvre et al. (2012)	SF1143	DY-751	6-FAM	101-147
csolfagus_29	Lefèvre et al. (2012)	FA29	BMN-6	HEX	124-158
csolfagus_19	Lefèvre et al. (2012)	FA19	DY-751	6-FAM	149-185
csolfagus_05	Lefèvre et al. (2012)	FA05	Cy5	ROX	156-194
csolfagus_06	Lefèvre et al. (2012)	FA06	BMN-6	6-FAM	197-225
FCM5	Tanaka et al. (1999)	MFC05	Cy5	6-FAM	277-333
Multiplex 2					
DUKCT_A_0	Lefèvre et al. (2012)	DUKCT	Cy5	ROX	76-114
EEU75_A_0	Lefèvre et al. (2012)	EEU75	DY-751	6-FAM	88-116
EMILY_A_0	Lefèvre et al. (2012)	EMILY	DY-751	6-FAM	139-185
EJV8T_A_0	Lefèvre et al. (2012)	EJV8T	Cy5	ROX	137-163
ERHBI_A_0	Lefèvre et al. (2012)	ERHBI	DY-751	6-FAM	157-192
concat14_A_0	Lefèvre et al. (2012)	CON14	Cy5	ROX	169-234
DZ447_A_0	Lefèvre et al. (2012)	DZ447	DY-751	6-FAM	173-219
DE576_A_0	Lefèvre et al. (2012)	DE576	BMN-6	HEX	219-240
<i>Picea abies</i>					
Multiplex 1					
SpAGG3	Pfeiffer et al. (1997)	SPAGG3	DY-751	HEX	104-166
paGB3	Besnard et al. (2003)	PAGB3	Cy5	ROX	98-150
WS0073.H08	Rungis et al. (2004)	WSH08	DY-751	FAM	186-244
UAPgGT8	Hodgetts et al. (2001)	UAPG8	Cy5	ROX	193-297
WS0092.A19	Rungis et al. (2004)	WSA19	BMN-6	HEX	207-251
WS0032.M17	Rungis et al. (2004)	WSM17	DY-751	HEX	249-270
PA44	Fluch et al. (2011)	PA44	Cy5	FAM	265-301
Multiplex 2					
WS0022.B15	Rungis et al. (2004)	WSB15	DY-751	FAM	159-217
WS0046.M11	Rungis et al. (2004)	WSM11	DY-751	FAM	204-236
PA51	Fluch et al. (2011)	PA51	Cy5	FAM	121-147

Marker name	Reference	Abbreviation (Online Resources 4 and 5)	Dye for Beckman Coulter CEQ8800	Dye for ABI 3730xl	Fragment length (bp), this study
EATC1E3	Scotti et al. (2002)	EA1E3	DY-751	ROX	118-177
SPAG2	Pfeiffer et al. (1997)	DPAG2	Cy5	FAM	88-140
EATC2G05	Scotti et al. (2002)	EA2G05	BMN-6	HEX	193-271
SPAGC1	Pfeiffer et al. (1997)	SPAGC1	BMN-6	HEX	74-128
EATC1B02	Scotti et al. (2002)	EA1B2	Cy5	ROX	177-228

PCR was performed using the Qiagen (Hilden) Multiplex PCR Kit and the following protocols:

- Beech, both multiplex sets identical - Total reaction volume 20 µl, 10µl Qiagen Multiplex PCR Master Mix 2x, 0.5µl of each primer with a concentration of 2 µM, and 1 µl of DNA. For amplification, we used the following protocol: 15 min 95 °C / 30 cycles (30 sec. 94 °C, 90 sec. 55 °C, 60 sec. 72 °C) / 30 min 60 °C.
- Spruce multiplex set 1: Reaction volume and composition identical to the conditions given for beech but with differing primer concentrations. Primer volumes are shown in table ***, original primer concentration 2 µM. Amplification conditions as follow: 15 min 95 °C / 30 cycles (30 sec. 94 °C, 90 sec. 55 °C, 30 sec. 70 °C) / 30 min 60 °C.
- Spruce multiplex set 2: Reaction volume and composition identical to the conditions given for beech but with differing primer concentrations. Primer volumes are shown in table ***, original primer concentration 2 µM. Amplification conditions as follow: 15 min 95 °C / 30 cycles (30 sec. 94 °C, 90 sec. 58 °C, 30 sec. 70 °C) / 30 min 60 °C.

Fragment analysis was done on Beckman Coulter CEQ8800 (AB Sciex) ABI and 3730xl (Thermo Fisher) sequencers. Allele calling was performed using GenomeLab (Beckman Coulter) and GeneMarker V3.0.1 (SoftGenetics).

Evaluation of errors

The appropriate variability of the markers and the sufficient sample sizes in our study ensure high reproducibility of the reconstructed pedigrees from repeated COLONY runs (see Data Analysis in Material and Methods). Mismatches in assigned parents between replicate runs were exemplarily estimated at a proportion of 0.2 % for seed samples and 0.9 % for natural regeneration samples. The proportion of assigned parent pairs with probabilities >0.95 was 99.3% for beech and 98.8% for spruce (including sampled and unsampled parents), pooled across all COLONY runs. Together with the re-estimated typing error rates of 0.5 % for beech and 0.6 % for spruce across all loci and all COLONY runs based on the best reconstructed pedigree, we conclude a high reliability of the collected genotype data.

References

- Besnard G, Acheré V, Faivre Rampant P, Favre J, Jeandroz S (2003) A set of cross-species amplifying microsatellite markers developed from DNA sequence databanks in *Picea* (Pinaceae). *Mol. Ecol. Notes* 3(3):380-383. <https://dx.doi.org/10.1046/j.1471-8286.2003.00456.x>
- Dumolin S, Demesure B, Petit RJ (1995) Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theor. Appl. Genet.* 91(8):1253-1256. <https://dx.doi.org/doi:10.1007/BF00220937>
- Fluch S, Burg A, Kopecky D, Homolka A, Spiess N, Vendramin GG (2011) Characterization of variable EST SSR markers for Norway spruce (*Picea abies* L.). *BMC Research Notes* 4(1):401. <https://dx.doi.org/10.1186/1756-0500-4-401>
- Hodgetts R, Aleksuk M, Brown A, Clarke C, Macdonald E, Nadeem S, Khasa D (2001) Development of microsatellite markers for white spruce (*Picea glauca*) and related species. *Theor. Appl. Genet.* 102(8):1252-1258. <https://dx.doi.org/10.1007/s00122-001-0546-0>
- Lefèvre S, Wagner S, Petit RJ, De Lafontaine G (2012) Multiplexed microsatellite markers for genetic studies of beech. *Mol. Ecol. Resour.* 12(3):484-491. <https://dx.doi.org/10.1111/j.1755-0998.2011.03094.x>
- Pastorelli R, Smulders MJM, Van't Westende WPC, Vosman B, Giannini R, Vettori C, Vendramin GG (2003) Characterization of microsatellite markers in *Fagus sylvatica* L. and *Fagus orientalis* Lipsky. *Mol. Ecol. Notes* 3(1):76-78. <https://dx.doi.org/10.1046/j.1471-8286.2003.00355.x>
- Pfeiffer A, Olivieri AM, Morgante M (1997) Identification and characterization of microsatellites in Norway spruce (*Picea abies* K.). *Genome* 40(4):411-419. <https://dx.doi.org/10.1139/g97-055>
- Rungis D, Bérubé Y, Zhang J, Ralph S, Ritland C, Ellis B, Douglas C, Bohlmann J, Ritland K (2004) Robust simple sequence repeat markers for spruce (*Picea* spp.) from expressed sequence tags. *Theor. Appl. Genet.* 109(6):1283-1294. <https://dx.doi.org/10.1007/s00122-004-1742-5>
- Scotti I, Magni F, Paglia G, Morgante M (2002) Trinucleotide microsatellites in Norway spruce (*Picea abies*): their features and the development of molecular markers. *Theor. Appl. Genet.* 106(1):40-50. <https://dx.doi.org/10.1007/s00122-002-0986-1>
- Tanaka K, Tsumura Y, Nakamura T (1999) Development and polymorphism of microsatellite markers for *Fagus crenata* and the closely related species, *F. japonica*. *Theor. Appl. Genet.* 99(1):11-15. <https://dx.doi.org/10.1007/s001220051203>