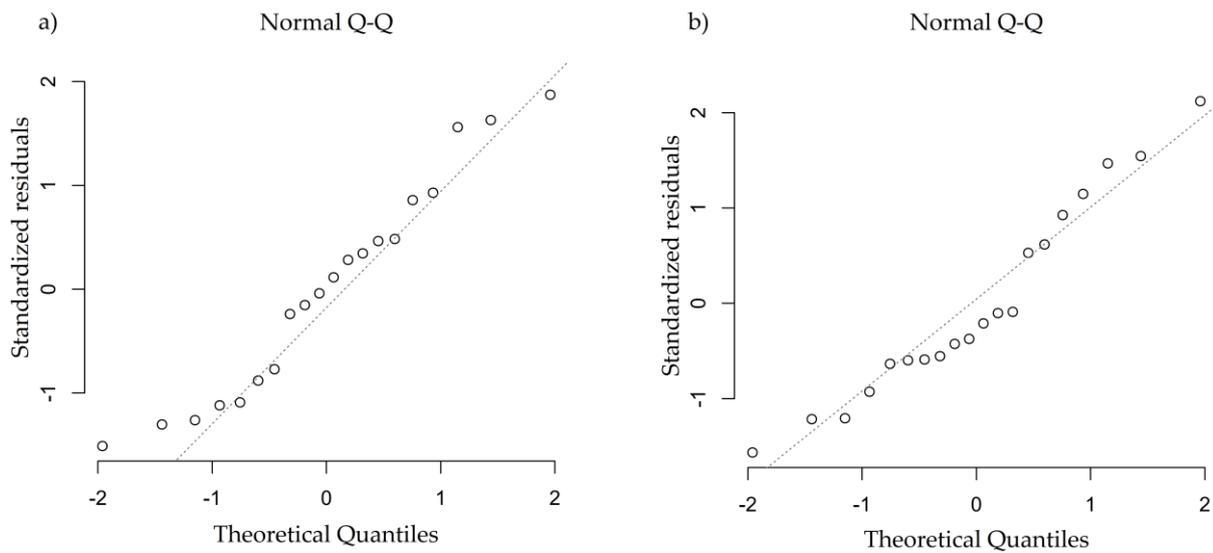


## SUPPLEMENTARY MATERIALS

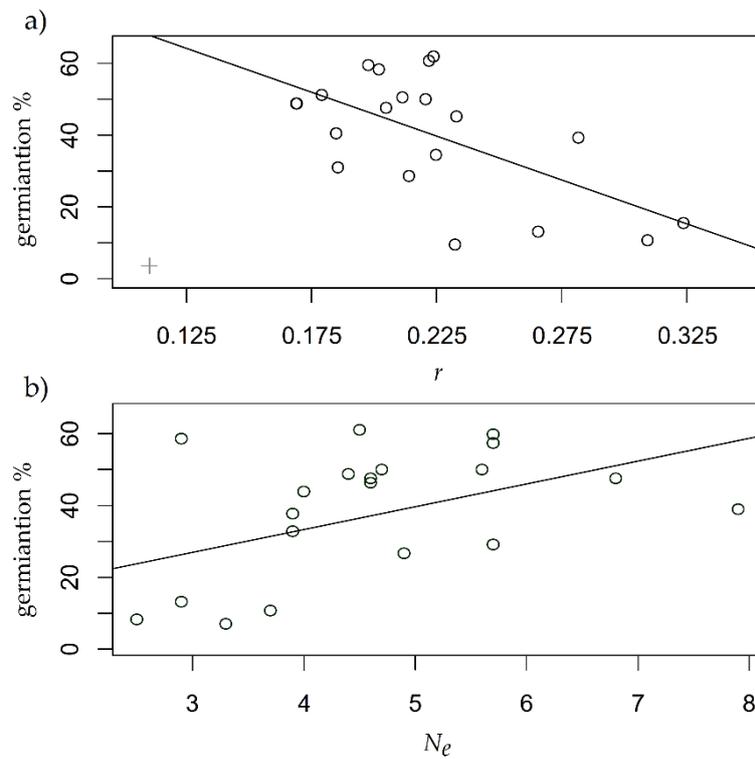
# Mating system in a native Norway spruce (*Picea abies* [L.] KARST.) stand - Relatedness and effective pollen population size show an association with the germination percentage of single tree progenies

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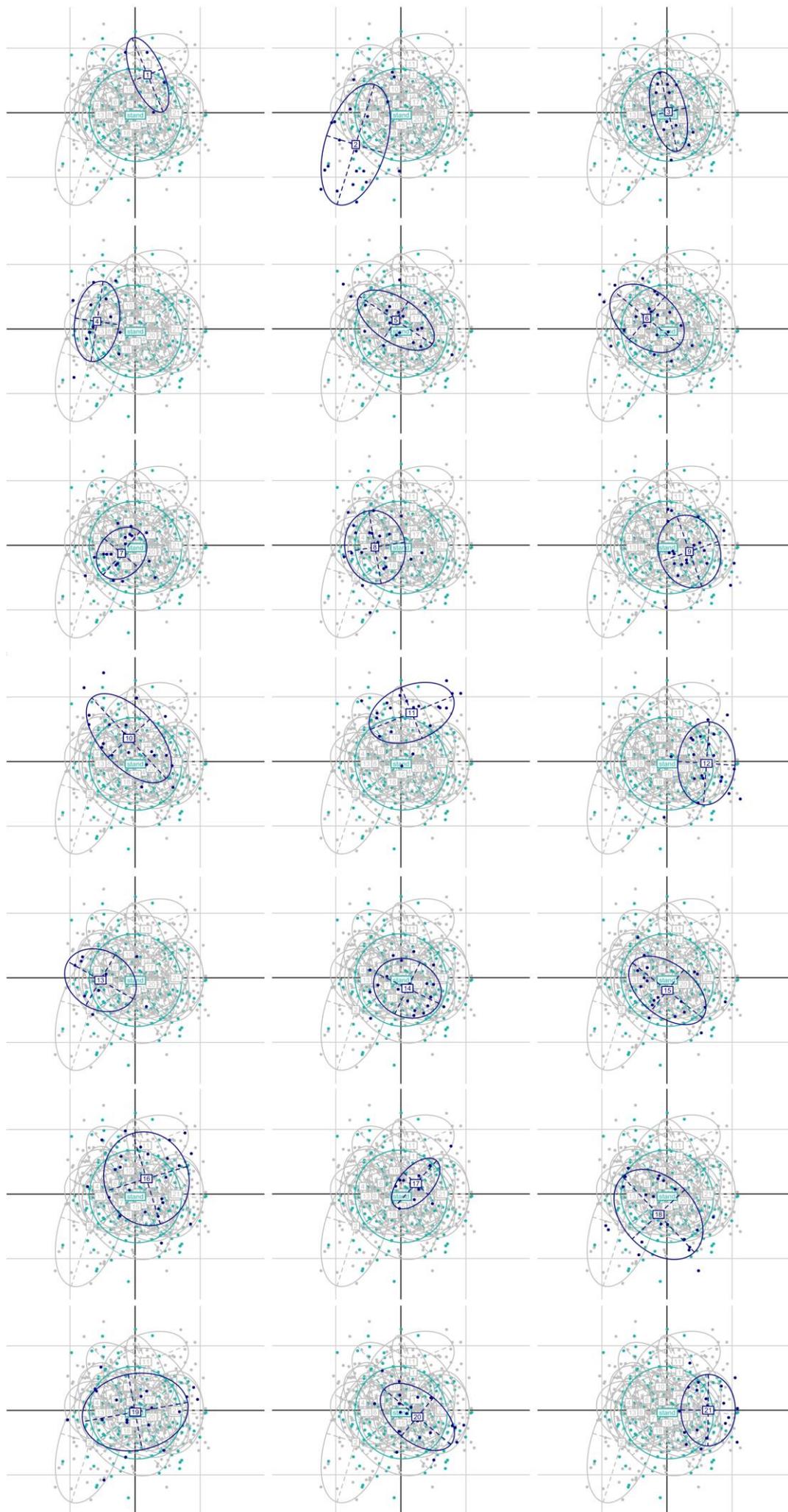
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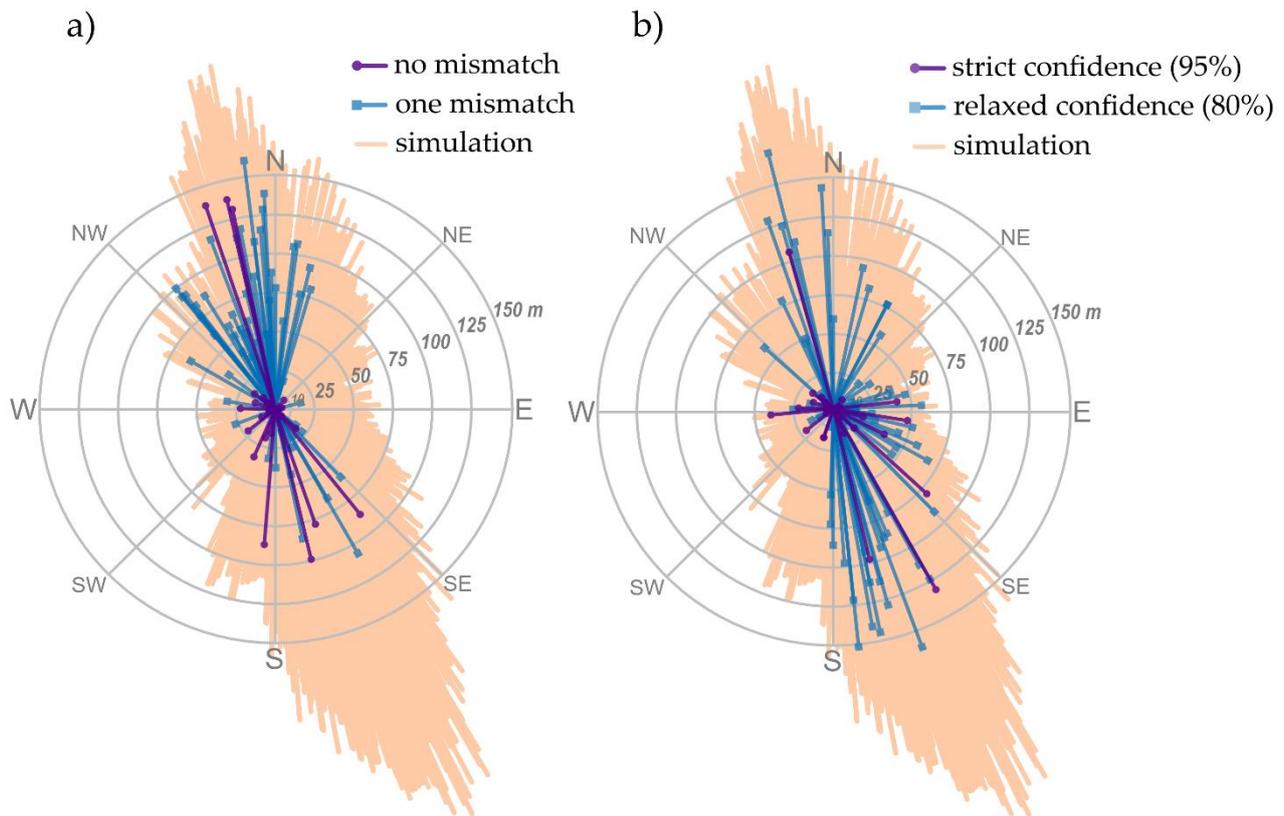
**Figure S1.** Quantile-Quantile diagram of the standardized residuals in the linear regression between within half-sib family seed germination rate presented in Figure 3 and a) rarefied mean relatedness or b) rarefied mean effective pollen population size.



**Figure S2.** Scatter plot of mean seed germination rate (y-axis) in 21 half-sib families plotted against the **a)** mean within half-sib family relatedness ( $r$ ) or **b)** effective population size ( $N_e$ ) of the half-sib families. The black line represents the corresponding linear regression trendline: **a)** adjusted  $R^2 = 0.34$ ,  $P = 0.004$  excluding half-sib family 1 (depicted by the grey cross) due to very limited sample size; **b)** adjusted  $R^2 = 0.15$ ,  $P = 0.046$ .



**Figure S3.** Principal Coordinate Analysis (PCoA) of embryos and also adult trees that represent potential pollen donors in the stand based on the pairwise Hamming distance [25] between them calculated using genotypes of 10 SSR markers. For better visualization of the embryos belonging to the same half-sib family, the figure presents the same PCoA plot 21 times, but each time with samples of a particular half-sib family highlighted by dark blue dots. Potential pollen donors in the stand are highlighted by turquoise, and all other datapoints are highlighted by grey. Distributions of the datapoints that belong to the same half-sib family are circled each by a 95% inertia ellipse.



**Figure S4.** Local pollen dispersal distances and angles of the assignment determined by **a)** matching the pollen haplotype with the genotyped individuals and **b)** using Cervus 3.0.7 software. All 21 seed-collected mother trees are projected to the centre of the plot. End-marked lines indicate the assigned pollen donor. **a)** A strict assignment with complete match for all markers and allowing a single mismatch for one of the markers are depicted by dark violet lines with round-pointed-ends and blue lines with square-pointed ends, respectively. **b)** A strict 95% confidence delta threshold and a relaxed 80% confidence delta threshold are depicted by dark violet lines with round-pointed-ends and blue lines with square-pointed ends, respectively. The simulated random mating events ( $N=20,000$ ) between 21 seed-collected mother trees and all genotyped trees are depicted by light orange lines in the background. Both observed and simulated data do not include pollen flow from outside the stand.

**Table S1.** SSR markers used for genotyping 200 adult trees including 21 seed-collected trees, embryos, and megagametophytes (Caré et al. [17], modified).

| SSR marker               | Allele size range, bp | Dye label (6-FAM and HEX) and the forward (F) and reverse (R) PCR primer nucleotide sequences (5'-3') | Repeat motif                                      | Reference            |
|--------------------------|-----------------------|---|---|----------------------|
| EATC1B2 <sup>a</sup>     | 197-219               | F: 6-FAM-TGGCATGAGATTTATGTGGTT<br>R: GTGTGCCACTCAACCTCAC  | (ATC)7(AT)3                                       | Scotti et al. [19]   |
| EATC1D2 <sup>a</sup>     | 180-236               | F: 6-FAM-TTGTTCATCGTCGTCATTGTC<br>R: TTTAGCCTCTGTTTTCTAGCG  | (ATC)3AT(ATC)6                                    |                      |
| EATC1E03 <sup>a</sup>    | 130-175               | F: 6-FAM-CCCCTTATTCCTAACGTCAAA<br>R: TACCAGTGGTGACAACGATG   | (CAT)4CGT(CAT)8CG<br>T(CAT)4CGT(CT)4CG<br>T(CAT)4 |                      |
| EATC2G05 <sup>a</sup>    | 193-254               | F: HEX-TGGAGCATGGGTAAATCG<br>R: TACCTCACACCCGTGAGAAT  | (AAT)5(CAT)16CAA(CAT)4                            |                      |
| PaGB3 <sup>b</sup>       | 109-150               | F: 6-FAM-AGTGATTAAACTCCTGACCAC<br>R: CACTGAATACACCCATTATCC  | (AT)11  | Besnard et al. [21]  |
| SpAG2 <sup>a</sup>       | 88-122                | F: 6-FAM-GCTCTTCACGTGTACTTGATC<br>R: TTCGAAGATCCTCCAAGATAC  | (TC)16  |                      |
| SpAGC1 <sup>a</sup>      | 71-121                | F: HEX-TTCACCTTAGCCGAGAACC<br>R: CACTGGAGATCTTCGTTCTGA  | (TC)5TT(TC)10                                     | Pfeiffer et al. [20] |
| SpAGG3 <sup>a</sup>      | 109-149               | F: HEX-AGCATGTTGTCCCATATAGACC<br>R: CTCCAACATTCCCATGTAGC  | (GA)24  |                      |
| WS00016.O09 <sup>b</sup> | 386-402               | F: HEX-CTTTGGGGGCTAGCAAGTTT<br>R: ATTCGGGCTTCATAGCACAA  | (AT)9   | Rungis et al. [22]   |
| WS00111.K13 <sup>b</sup> | 212-272               | F: HEX-GACTGAAGATGCCGATATGC<br>R: GGCCATATCATCTCAAATAAAGAA  | (AT)9   |                      |

<sup>a</sup> gSSRs, <sup>b</sup> EST-SSRs

**Table S2.** Concentration ( $c_i$ ,  $\mu\text{M}/\mu\text{l}$ ) of each forward and reverse primer in the multiplex reactions (Caré et al. [17], modified).

| Multiplex 1        |       | Multiplex 2     |       | Multiplex 3     |       | Multiplex 4        |       |
|--------------------|-------|-----------------|-------|-----------------|-------|--------------------|-------|
| SSR                | $c_i$ | SSR             | $c_i$ | SSR             | $c_i$ | SSR                | $c_i$ |
| <i>WS00111.K13</i> | 3.57  | <i>SpAGC1</i>   | 2.14  | <i>EATC1E03</i> | 1.25  | <i>SpAGG2</i>      | 1.79  |
| <i>EATC1D2</i>     | 2.14  | <i>EATC2G05</i> | 2.14  | <i>SpAG2</i>    | 1.79  | <i>WS00016.O09</i> | 3.57  |
|                    |       | <i>EATC1B2</i>  | 1.07  | <i>SpAC1F07</i> | 3.57  | <i>PaGB3</i>       | 1.07  |

**Table S3.** PCR touch-down protocol used for amplification of SSR-markers.

| Step            | Temperature, °C | Time, min         | Cycle repeats |
|-----------------|-----------------|-------------------|---------------|
| Incubation      | 95              | 15                | 1             |
| Touch-down      | Denaturation    | 94                | 1             |
|                 | Annealing       | 60 ( $\Delta$ -1) | 1             |
|                 | Extension       | 72                | 1             |
| Denaturation    | 95              | 1                 | 25            |
|                 | Annealing       | 50                |               |
|                 | Extension       | 72                |               |
| Final extension | 72              | 20                | 1             |

**Table S4.** Pearson’s correlation coefficient (PCC) for the comparisons (1 vs. 2) of mean within half-sib families relatedness estimated with different algorithms and reference allele frequencies.

| Calculation 1              |   | Calculation 2              |   | PCC      |
|----------------------------|---|----------------------------|---|----------|
| Algorithm 1 <sup>(1)</sup> | reference allele frequency 1 <sup>(2)</sup> | Algorithm 2 <sup>(1)</sup> | reference allele frequency 2 <sup>(2)</sup> |          |
| triadic likelihood         | default                                     | triadic likelihood         | stand & embryo                              | 0.996*** |
| triadic likelihood         | default                                     | triadic likelihood         | stand                                       | 0.979*** |
| triadic likelihood         | stand & embryo                              | triadic likelihood         | stand                                       | 0.991*** |
| efficient method-of-moment | default                                     | efficient method-of-moment | stand & embryo                              | 0.956*** |
| efficient method-of-moment | default                                     | efficient method-of-moment | stand                                       | 0.734*** |
| efficient method-of-moment | stand & embryo                              | efficient method-of-moment | stand                                       | 0.794*** |
| dyadic likelihood          | default                                     | dyadic likelihood          | stand & embryo                              | 0.998*** |
| dyadic likelihood          | default                                     | dyadic likelihood          | stand                                       | 0.981*** |
| dyadic likelihood          | stand & embryo                              | dyadic likelihood          | stand                                       | 0.992*** |
| triadic likelihood         | default                                     | efficient method-of-moment | default                                     | 0.692*** |
| triadic likelihood         | default                                     | dyadic likelihood          | default                                     | 0.983*** |
| efficient method-of-moment | default                                     | dyadic likelihood          | default                                     | 0.627**  |
| triadic likelihood         | stand & embryo                              | efficient method-of-moment | stand & embryo                              | 0.683*** |
| triadic likelihood         | stand & embryo                              | dyadic likelihood          | stand & embryo                              | 0.988*** |
| efficient method-of-moment | stand & embryo                              | dyadic likelihood          | stand & embryo                              | 0.632**  |
| triadic likelihood         | stand                                       | efficient method-of-moment | stand                                       | 0.4      |
| triadic likelihood         | stand                                       | dyadic likelihood          | stand                                       | 0.988*** |
| efficient method-of-moment | stand                                       | dyadic likelihood          | stand                                       | 0.345    |

<sup>(1)</sup>triadic likelihood described in [30]; efficient-method-of moment described in [32]; dyadic likelihood described in [33] <sup>(2)</sup>reference allele frequency used in the calculation; default uses the sample data for estimating the population allele frequency, here the embryo data; stand & embryo is the combined allele frequencies of the sampled stand and the embryos and stand is the adjusted allele frequencies of only the stand data; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Table S5.** Adjusted R<sup>2</sup> of linear regressions between the germination percentage and the mean within family relatedness estimated with different algorithms and reference allele frequencies from the original data, excluding half-sib-family 1 due to very limited sample size.

| Algorithm <sup>(1)</sup>   | reference allele frequency <sup>(2)</sup> | adjusted R <sup>2</sup> |
|----------------------------|---|-------------------------|
| triadic likelihood         | default                                   | 0.3435**                |
| triadic likelihood         | stand & embryo                            | 0.3009**                |
| triadic likelihood         | stand                                     | 0.2396*                 |
| efficient method-of-moment | default                                   | 0.5326***               |
| efficient method-of-moment | stand & embryo                            | 0.446**                 |
| efficient method-of-moment | stand                                     | 0.08051                 |
| dyadic likelihood          | default                                   | 0.2504*                 |
| dyadic likelihood          | stand & embryo                            | 0.2137*                 |
| dyadic likelihood          | stand                                     | 0.1552*                 |

<sup>(1)</sup>triadic likelihood described in [30]; efficient-method-of moment described in [32]; dyadic likelihood described in [33] <sup>(2)</sup>reference allele frequency used in the calculation; default uses the sample data for estimating the population allele frequency, here the embryo data; stand & embryo is the combined allele frequencies of the sampled stand and the embryos, and stand is the adjusted allele frequencies of only the stand data; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .