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Clonality in black locust (*Robinia pseudoacacia* L.) and implications for seed production

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Abstract

Key message The strong clonal growth of black locust (*Robinia pseudoacacia* L.) not only influences the stand structure of natural or artificially established stands, but also the genetic composition of seed harvested from such clonal stands. For the commercial production of genetically diverse seeds, the stand structure should be taken into account or, at best, seeds harvested from seed orchards should be used.

Context Black locust is characterised by intensive asexual reproduction through the formation of root suckers. By this means clonal structures can develop within black locust stands, in which ramets of a single clone can dominate extensive areas.

Aims We want to analyse to what extent clonal structures within black locust stands negatively influence the genetic composition and diversity in seed harvested in such stands. We discuss how a potential reduction in genetic diversity can be reduced by measures taken during harvesting and whether the harvesting of seed orchards may be a better alternative.

Methods We compare the genetic composition and diversity of parent trees and seed harvested from a clonal black locust stand with a seed orchard in which multiple ramets of selected clones were arranged in a randomised design.

Results Within the clonal stand, parent contributions to the seed lot analysed proved to be strongly uneven. Selfing rates were high and large full-sib families dominated within the seed lot. Although the relatively strong pollination from unknown pollen donors, probably located outside of the stand, prevented a massive loss of alleles, high selfing rates and the formation of large full-sib families led to an unequal distribution of alleles within the progeny. Within the seed orchard—even though it had a lower number of clones than expected—the randomised design promoted a more diverse pollination pattern.

Conclusion We conclude that for black locust, seed orchards have the greater potential to ensure a balanced genetic composition of harvested seed lots. If economic considerations make it necessary to harvest seed stands, this should only be done in a considered manner and, if possible, with knowledge of the clonal structures of the stand.

Keywords Black locust, Clonality, Seed orchard, Genetic diversity, Forest Reproductive Material (FRM), SSR markers

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1 Introduction

Black locust (*Robinia pseudoacacia* L.) is a deciduous tree of the Fabaceae family. Native to eastern North America (Little and Viereck 1971; Huntley 1990), it was introduced to Europe probably in the seventeenth century and is now naturalised in many parts of Europe (Sitzia et al. 2016; Martin 2019; Nicolescu et al. 2020). The origin of the black locust populations in Europe can be traced back to populations originating from the Appalachian Mountains (Bouteiller et al. 2019).

Black locust is diploid with 2n = 22 chromosomes. A chromosome-level genome assembly of 682.4 Mb has recently been published (Wang et al. 2023). Sexual reproduction of black locust occurs via insect pollination of its hermaphrodite flowers (Huntley 1990). A total of 5–8 seeds are produced in long dark brown pods (Martin 2019). However, natural regeneration from seeds is rare (Nicolescu et al. 2020), natural seed dispersal is constrained by rather heavy seeds (Vítková et al. 2017) and natural reproduction is primarily asexual through the formation of root suckers and, to a much lesser degree, stool shoots. Root suckers are produced abundantly, especially after disturbance/damage to the root system. By this means, dependent on the stand history, clonal structures in which ramets of a single clone dominate extensive areas, can develop within a stand (Nicolescu et al. 2020).

The wood of black locust is durable and rot-resistant (Pollet et al. 2008) and has become a popular multipurpose wood in Europe (Sitzia et al. 2016; Nicolescu et al. 2020). Black locust is seen as a valuable tree for future forestry and can also be used for biomass production via simple coppice, due to its relatively fast and clonal growth (Grünewald et al. 2009; Sitzia et al. 2016; Vítková et al. 2017; Nicolescu et al. 2020). However, some European countries list black locust as an invasive species due to its ability to spread rapidly (Nicolescu et al. 2020). Once planted, black locust can hardly be eliminated from an area since injuries, e.g. through clearcutting, favour the formation of new root suckers and stool shoots. Moreover, black locust can change existing soil conditions through nitrogen fixation (Sitzia et al. 2016; Vítková et al. 2017). This di-nitrogen fixation happens through symbiotic *Rhizobium* bacteria associated with the root nodules. Through the later mineralisation of leaf litter, this process makes nitrogen available to other plants (Sitzia et al. 2016). This can have a positive effect on plant growth. However, nitrogen fixation can significantly and irreversibly change the soil conditions, which can ultimately alter the existing ecosystem and suppress species communities adapted to poor soil conditions (Benesperi et al. 2012; Vítková et al. 2017).

The importance of black locust cultivation in Europe varies considerably from country to country. In Hungary, for example, black locust cultivation is of great importance and black locust is cultivated on over 400,000 ha, which equals more than 20% of the total forest area of the country (Rédei et al. 2011). In Romania, black locust is grown on about 250,000 ha (Enescu 2013). In Germany, black locust is cultivated on about 42,200 ha of forest land, the majority (about 24,600 ha) located on dry and sandy soils in the eastern states of Brandenburg and Saxony-Anhalt (data according to the German National Forest Inventory 2012, available on https://bwi.info).

In Germany, black locust—since 2003—is subject to the Act on Forest Reproductive Material (Anonymous 2002a, b). The production and trade of reproductive material are limited to material originating from selected or tested seed stands, qualified or tested seed orchards and tested clones. For seed stand approval (category "selected") the minimum requirements are a stand age of at least 30 years and a number of at least 20 adult trees. Seed collection has to be carried out from at least 10 different trees in order to obtain seeds with sufficient genetic diversity. However, the regulations do not specify a minimum distance between trees used for seed harvest or a minimum stand size for black locust seed stands. In comparison, for poplar (*Populus* sp.), also a deciduous tree species with a high ability for clonal growth, a minimum area of 0.25 ha has been specified. Currently, a total of four seed orchards covering an area of 2.2 ha in total and 37 seed stands with a total area of 112 ha exist in Germany, of which an average of 260 kg of seeds per year (2003–2020) have been harvested. About 10% of the total seed volume originates from seed orchards (Schirmer and Cremer 2020).

This study deals with the clonal growth in black locust stands and its implications for seed production. Extensive clonal structures might negatively influence the genetic intermixture and the preservation of genetic diversity within a seed lot harvested from such a stand. However, a high level of genetic diversity is crucial to allow a species to adapt to environmental changes, and commercially traded seeds should not only inherit selected phenotypic traits, but also include a preferably high level of genetic diversity, which is important for the formation of adaptive future stands, able to cope with the challenges of global climate change, pollution or new pathogens (White et al. 2007; Ivetic et al. 2016; Ingvarsson and Dahlberg 2019).

To analyse the potential implications of clonal structures for the genetic composition of a seed lot harvested from a clonal stand, a test seed harvest was carried out in a potentially clonal black locust stand. The conditions for the harvest were based on the requirements of

the German Act on Forest Reproductive Material. Since the law does not specify a minimum stand size for black locust seed stands, the minimum size of 0.25 ha specified for poplar has been used for orientation.

An area of the selected stand corresponding to this size was sampled and genotyped to allow an overview of the clonal structure of the stand. A tree-wise seed harvest of 36 seed trees located within this area was carried out to analyse the genetic composition of the seeds obtained. The aim of this study was to investigate whether a potentially "patchy" clonal structure in the black locust stand would lead to a certain genetic uniformity of the harvested seed trees and to what extent the potential clonal structure of the stand influences the selfing rate, the number and genetic contribution level of different parent clones to the production of the progeny, and the genetic composition and diversity of the harvested seed.

For comparison, a black locust seed orchard was examined. The seed orchard analysed shows an artificial "intermingled" structure of known clones. Does this distribution lead to a better genetic intermixture of all parents in the production of the progeny and a more variable genetic composition and higher genetic variety of the harvested seed lot? What recommendations can be made for the future seed production of black locust and in more general terms other clonal tree species?

2 Material and methods

2.1 Sampling

The stand "Gottesgabe" (N 52.6301, E 14.1633) is located near Gottesgabe (Neuhardenberg, Brandenburg, Germany). It covers a total area of about 3 ha (approx. 300 m × 100 m), surrounded mainly by Scots pine (*Pinus sylvestris* L.). Other black locust trees grow in the immediate vicinity. An estimated 20–30% of the surrounding trees are black locust. A larger black locust stand is located approx. 400 m to the west of the stand.

The stand "Gottesgabe" was established in 1982 out of a stand consisting of Scots pine (average age about 90 years) and black locust (average age about 60–70 years). The original stand was subject to a clear-cut and stripe-wise ploughing to stimulate the formation of black locust root suckers. After one year the stool shoots were removed and only plants derived from root suckers were left. The stand was thinned to 4900 trees/ha after four years and to 1850 trees/ha after 18 years.

A first sampling was conducted in an area of about 0.25 ha, located in the south-western corner of the stand. The 0.25 ha corresponds to the minimum stand size specified for poplar, a comparable tree species with clonal growth, by the German Act on Forest Reproductive Material (Anonymous 2002a, b). Within this area, an almost complete sampling of all trees with a minimum diameter at breast height (DBH) of 7 cm was carried out. Relative positions (distance and angle) of 240 trees were measured using a "Ledha Geo" instrument (Jenoptik AG, Jena, Germany). The mean distance between trees was 2.3 m. The mean height of the trees was 14.9 m (range 8.4–18.8 m), and the mean DBH was 14.5 cm (range 7.3–28.2 cm). Sampling was performed using a pole pruner. Leaf samples for genotyping were obtained for 218 trees. For 22 trees, no leaf samples could be obtained. Seed trees were selected based on the accessibility of seeds from the ground. Seeds from 36 seed trees were harvested. The average distance from one seed tree to the nearest seed tree was 5.6 m (range: 1.1–16.7 m).

Seeds were germinated in the greenhouse. Leaves from 375 seedlings were collected and stored at − 20 °C until DNA extraction. For seed trees with no or very few germinated offspring individuals, a total of 91 additional seeds of the respective trees were soaked in water overnight. Then, embryos were dissected from the soaked seeds and used for DNA extraction.

In a second partial sampling approach, 92 individuals located in the remaining approximately 2.75 ha of the stand were sampled. Trees were chosen due to dominance and representative distribution across the stand. The mean distance between sampled trees was 8.8 m. GPS coordinates (± 2–5 m) of all sampled trees were measured using a handheld GPS receiver.

In addition, a total of 16 trees were sampled from two patches located approx. 200 m (3 trees) and 400 m (13 trees, part of a larger black locust stand) to the west of the stand.

The seed orchard "Graeff" (N 51.5027, E 13.4228) is located near Zeischa (Bad Liebenwerda, Brandenburg, Germany). It was established by a private owner using clones selected as plus trees from four semi-natural source stands, located near Buckow, Sauen and Großbeeren in central Brandenburg, as well as Welzow in southern Brandenburg. The next black locust trees outside of the seed orchard are located at a distance of approximately 300 m. The seed orchard design is based on double rows (1.5 m resp. 2 m, alternate spacing) and a distance of 4 m between plants within the rows. The influence of weeds and drought and the formation of root suckers is reduced by using ground cover. Branches are arranged in a horizontal espalier-like manner to promote flowering and facilitate seed harvest (Fig. 1). The establishment of the seed orchard has been described by Naujoks et al. (2012). At the time of sampling, the seed orchard consisted of 6 rows with 20 grafted plants each (= 120 plants).

According to the planting scheme, the seed orchard included 24 different clones (3–6 ramets each). For 12 of these clones, ramets were produced by grafting of scions

Fig. 1 Seed orchard "Graeff" with ground cover and espalier-like arrangement of branches (photo: R. Becker, 2012)

of selected trees on seedling-rootstocks. For the other 12 clones, ramets were produced by microvegetative propagation (described by Boine et al. (2008).

Leaf samples from all 24 clones were collected and stored at – 20 °C until DNA extraction. Seeds were harvested from 29 seed trees randomly distributed over the seed orchard. According to the planting scheme, the harvested seed trees represented 16 different clones. For the remaining eight clones no seeds were available. For DNA extraction seeds were germinated in a greenhouse to obtain leaf samples or embryos were dissected from seeds soaked in water. A total of 669 offspring individuals were used for DNA extraction.

2.2 DNA extraction and marker analysis

DNA extraction was carried out from leaves or embryos according to a modified CTAB protocol, described by Dumolin et al. (1995). Genotyping was based on 12 nuclear SSR markers developed by Lian and Hogetsu (2002) (Rops 02, 05, 06, 08 and 16) and Mishima et al. (2009) (RP 01B, 032, 035, 106, 109, 200, 206) organised in two multiplex sets described by Liesebach and Ewald (2012). PCRs were performed as described in Liesebach and Ewald (2012) using the "Multiplex PCR Kit" from Qiagen (Hilden, Germany). Fragment separation was carried out on a Beckman Coulter CEQ 8000 capillary sequencer (Beckmann-Coulter, Brea, USA). Fragment analysis and genotyping were carried out using the CEQ 8000 Fragment Analysis software (Beckmann-Coulter, Brea, USA).

2.3 Data analysis

The data set used for data analysis is available online on OSF (https://osf.io/7xrky/, Pakull et al. (2024)). Individuals with more than 30% missing data were excluded from further genotype analysis. Pedigree reconstruction, identification of full and half sibships and genotype reconstruction of unsampled parents and determination of a sibship-based effective population size (Ne) under random and non-random mating models were performed using the software COLONY (Version 2.0.6.6, Jones and Wang (2010)). Rates of dropouts/null alleles and mistyping were adjusted according to values estimated by COLONY. Analyses were run using the following parameters: known maternal sibship, female and male polygamy, monoecious and diploid species, length of run: medium, analysis method: FL-PLS combined (FL: full likelihood, PLS: pairwise-likelihood score), weak prior. All other parameters were set to default. For each stand the analysis was repeated at least three times with different random seed numbers to verify the results.

Standard population genetic parameters (observed (Ho) and expected heterozygosity (He), mean number of alleles per locus (A) and mean effective number of alleles (Ae)) were calculated using GenAlEx (Peakall and Smouse 2012). Evenness (E), a parameter that measures the similarity of the observed distribution of allele frequencies to an equal distribution of alleles (Gregorius 1990) and the genetic distance between the different generations (Nei 1972) was calculated with GDA-NT 2021 (Degen 2022). Boxplots were drawn using R 4.4.0 (RCoreTeam 2023), Rstudio (RStudioTeam 2023) and the ggplot2-package (Wickham 2016). To test the significance of potential changes of the population genetic parameters between the parental and offspring generation in "Gottesgabe" and "Graeff", we performed unpaired two-sided *t*-tests. The Kolmogorov–Smirnov test was used to check for normality. Both analyses were carried out using R 4.4.0, Rstudio and the olsrr-Package (Hebbali 2023). Linkage disequilibrium between the different marker combinations was analysed using the web version of Genepop (Raymond and Rousset 1995; Rousset 2008).

3 Results

3.1 Stand "Gottesgabe": clonal structure and pollination pattern

Genotype analysis of 218 adult trees collected during the almost complete sampling of approximately 0.25 ha resulted in the identification of a massive clonal structure of the analysed stand. All 218 individuals represented only two different genotypes (clones A and B). Ramets of the respective clones were located in two clearly defined spatial clusters. Out of the 36 seed trees sampled for seed collection, 22 were ramets of clone A and 14 were ramets of clone B. This means that all analysed offspring individuals genetically descend from only two different mothers. Figure 2A shows a map of the trees sampled within the first sampling approach.

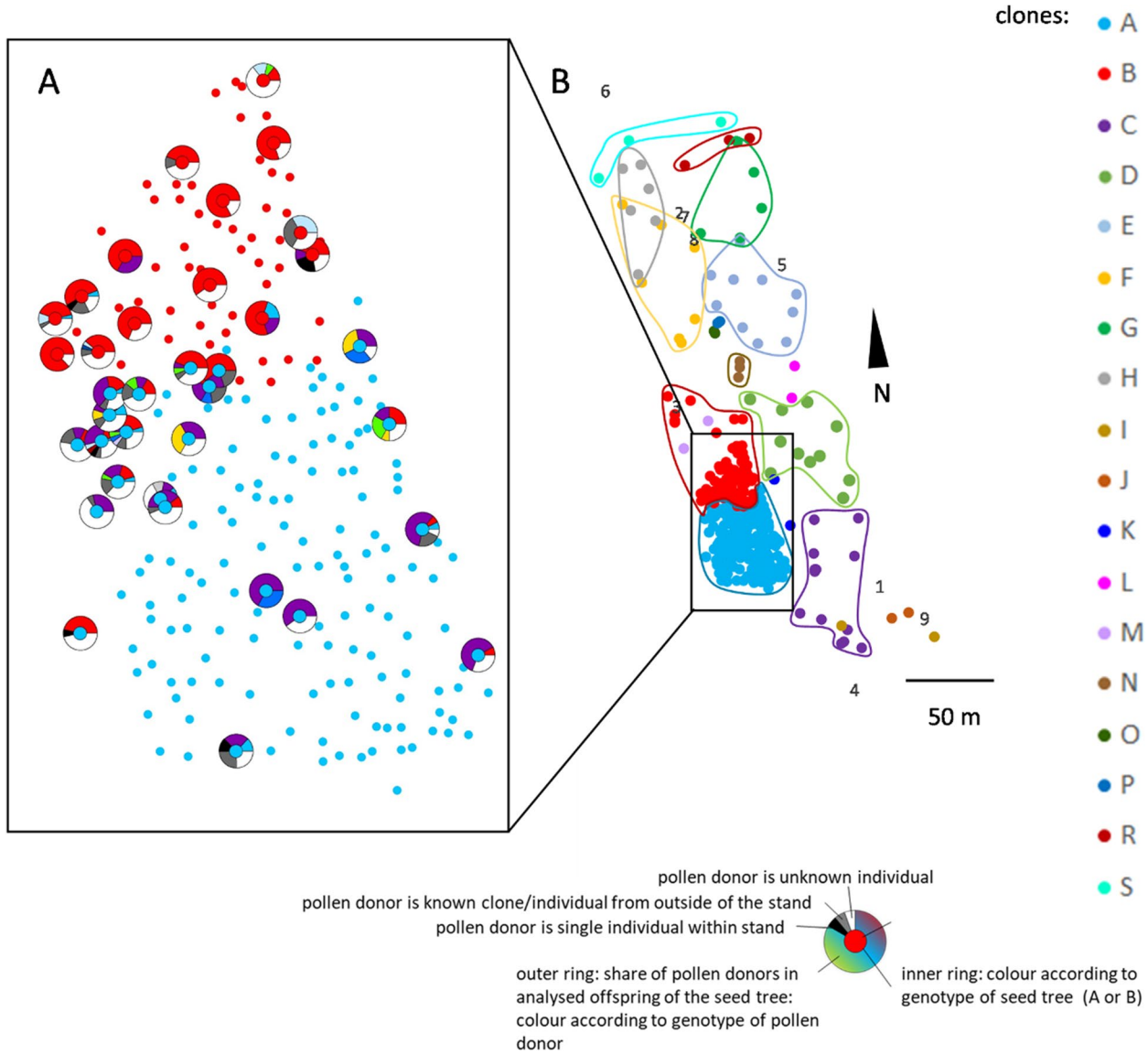

Fig. 2 Stand composition of the stand "Gottesgabe": **A** spatial positions of 218 trees collected during sampling in an area of about 0.25 ha located in the south-western corner of the stand. Ramets of the two identified genotypes are shown as blue (clone A) or red (clone B) dots. Pie charts show the share of different pollen donors in the analysed offspring of the seed trees used for seed harvest (colours according to clone colours in Fig. 2B, see legend in the bottom right corner. **B** Spatial positions of all 310 trees sampled within the stand "Gottesgabe". Each tree is represented by a single dot. Dot colour indicates the different genotypes/clones (see legend on the right). Nine single individuals (only one individual of this genotype has been identified/sampled) are shown as Numbers 1–9. Areas with at least 3 identical ramets are outlined in different colours to illustrate the "patchy" arrangement of the ramets of different clones

A second sampling approach was carried out to further analyse the clonal structure of the whole stand and to allow the identification of additional pollen donors for the analysed progeny. In a representative sampling, 92 dominant trees were sampled within the remaining approximately 2.75 ha of the stand. Figure 2B indicates the position of all trees sampled within the stand "Gottesgabe".

This second sampling confirmed a clonal, spatially clustered structure of the stand. A total of 18 clonal genotypes (2 to 154 identified ramets per genotype) and 9 single genotypes were identified within the 310 trees analysed. Genotypes with a higher number of ramets show that a "patchy" clone distribution with defined spatial clusters of the different genotypes can be observed throughout the whole stand.

Successful pedigree reconstruction was performed with COLONY for 433 offspring individuals (seeds/seedlings) from 36 seed trees. Pie charts for each seed tree show the share of different pollen donors in the offspring of the respective seed tree (Fig. 2A).

Looking at the pollination ratios shown in Fig. 2A, the high proportion of selfing that can be found in the analysed offspring of clone B is striking. A total of 48.7% of all analysed seeds of clone B seed trees descend from selfing. In comparison, a selfing share of only 3.6% was found for the offspring of clone A seed trees. The combination of both selfing rates leads to a total selfing rate of 19.4% in the progeny.

The offspring of clone A was predominately fathered by either clone C (25.3%) or clone B (18.2%). The clusters of these two clones are the northern and eastern neighbours of the clone A cluster. The predominant role of neighbouring genotypes as pollen donors is to be expected in an insect-pollinated species. However, the neighbouring cluster clones for clone B, clone A in the south and clone D in the east only account for only 2% (clone A) and 0.7% (clone D) of the pollen donors of clone B seed trees.

Other clones/individuals identified within the analysed stand play only minor roles as pollen donors for both types of mother clones.

Pollination by unknown pollen donors (either from individuals located outside of the stand or from some individuals with unsampled/undiscovered genotypes located within the stand) played a significant role and was found for about 34% for both mother clones (34.5% for clone A and 34.2% for clone B). A total of 59 to 60 potential genotypes of unsampled fathers were inferred by COLONY. Pollination by genotyped clones/individuals located outside of the stand within the two patches of black locust sampled in approx. 200 m (3 trees, 2 different clones/genotypes) and 400 m (13 trees, 3 different clones/genotypes) distance was found for 1.4% (clone A) and 0.7% (clone B) for the patch at 200 m distance and for 7.8% (A) and 4.6% (B) for the patch at 400 m distance.

A mating scheme based on the absolute numbers of offspring (281 offspring individuals of clone A seed trees and 152 offspring individuals of clone B seed trees, Appendix: Fig. 5) shows the very uneven contribution of the different clones to seed production. This is of course highly influenced by the unintended harvest of seeds from only two genetically different mother clones due to the massive clonal structure of the stand. However, the majority of clones located within the stand do not play a major role as pollen donors for clone A or B either.

3.2 Seed orchard "Graeff": clonal composition and pollination pattern

According to the planting scheme the seed orchard consisted of 24 different clones (3–6 ramets each). However, during genotyping only 13 different clones were identified. The reduction in clone/genotype number is based on the fact that different plus trees selected from the same source stand turned out to be genetically identical. The apparently high clonal structure of black locust stands (as observed in the stand "Gottesgabe") potentially led to the selection and grafting/propagation of different plus trees which were actually ramets of the same clone. Since this multiple selection did not occur evenly over all four source stands (stand "Hasenholz": 10 selected plus trees correspond to 4 genetic clones; "Sauen": 2 plus trees, 1 clone; "Welzow": 8 plus trees, 6 clones; "Grossbeeren": 4 plus trees, 2 clones) the number of ramets per clone is very uneven and varies between 3 and 26 (see Table 1).

Figure 3 gives a schematic overview of the clone composition of the seed orchard. One additional single genotype (N) probably represents an overgrowing rootstock. Seed harvest was performed from 30 seed trees, which did not—as originally assumed—correspond to 16 but only to 10 different clones and the single potential rootstock genotype. For three clones (clones C, G and H), no seed was available on any ramet.

The number of ramets per clone used for seed harvest and the number of analysed offspring individuals per mother clone varies. The exact numbers are listed in Table 1. However, the share of different mother clones in the total harvest is much more diverse than in "Gottesgabe".

Pedigree reconstruction with COLONY was performed for a total of 581 offspring individuals. Figure 6 (Appendix) shows the mating scheme of the seed orchard "Graeff".

A pollinator from within the seed orchard could be identified for 91.9% of the progeny. This corresponds to a total rate of pollination from unknown pollen donors (from outside of the seed orchard) of 8.1%. A total of 13 to 16 potential genotypes of unknown fathers were inferred by COLONY. The clones C, G and H did not contribute any pollen to the offspring generation. Together with the non-availability of seeds from these clones reported above, this indicates that these clones have not flowered.

Otherwise, the genetic contribution of the different clones to the progeny is more diverse than in "Gottesgabe", but still remains unevenly distributed. This is partly due to the uneven seed harvest from the different clones, which was influenced by the genetic identity of clones originally thought to be different. The total selfing rate is 7.1%. However, selfing rates differ between the different clones. In particular, clone K (22.9%) and

Table 1 Seed trees used for seed harvest in the seed orchard "Graeff"

Clone/genotype	No of ramets in seed orchard	No of ramets used for seed harvest	Total No of analysed offspring individuals per mother clone
A	26	3	60
B	16	2	31
C	9	0 (not flowering)	0
D	10	3 (1 ramet: single offspring only)	29
E	11	5	102
F	15	6	122
G	6	0 (not flowering)	0
H	4	0 (not flowering)	0
I	5	2	58
J	3	1	27
K	3	2	35
L	5	2	34
M	6	3	64
N	1 (rootstock)	1	19

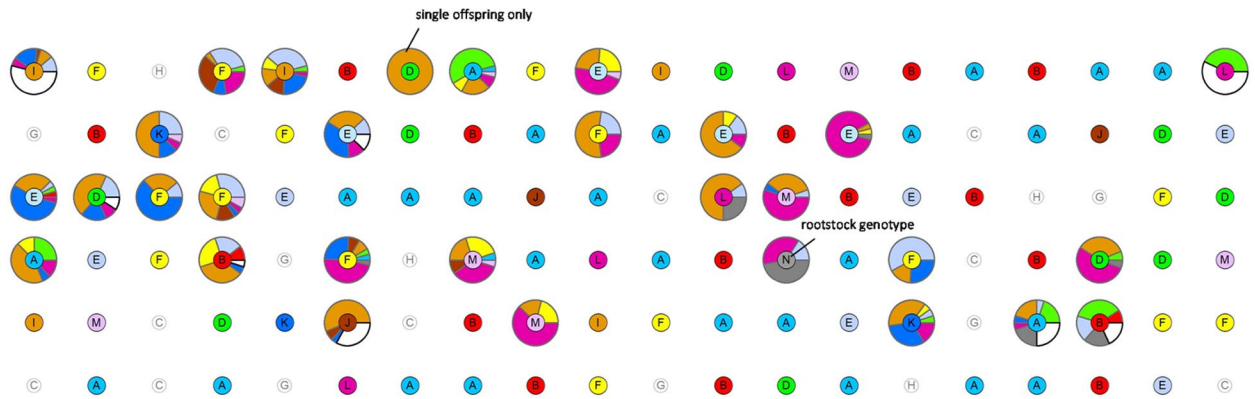

Fig. 3 Schematic plan of the seed orchard "Graeff" (not to scale). The seed orchard contains 6 rows with 20 grafted plants each (= 120 plants). Each plant is represented by a dot, clone name (A-N) is given on top of the dot. Please note: clones of the same name and colour in "Gottesgabe" and "Graeff" are not genetically identical. Clones C, G and H did not flower and are shown in white and light grey. For the trees used for seed harvest, pie charts show the share of different pollen donors in the offspring of the respective seed tree. Colours for the pollen donors used in the pie charts are in correspondence with the colours used for the adult clones of the seed orchard. White colour in the pie charts represents the share of pollen from unknown fathers. Pollen from selfing events is shown in the same colour as the respective seed tree. Only one offspring individual from clone D in row 1 was analysed. Clone N in row 4 represents the potential rootstock genotype

the potential rootstock genotype N (47.4%) show an increased selfing rate.

3.3 Comparison of the genetic composition of the progeny of the stand "Gottesgabe" and the seed orchard "Graeff"

As can be seen by comparing the mating schemes, the genetic contribution of the different adult clones to the progeny varies between the stand "Gottesgabe" and the seed orchard "Graeff". This becomes even clearer when directly comparing the contributions of ovules, pollen and total gametes (ovules plus pollen) of the individual clones in "Gottesgabe" and "Graeff" (Fig. 4).

Figure 4 shows the share of the individual parent clones in ovule, pollen and total gamete production. For the stand "Gottesgabe" the ovule, pollen and gametic

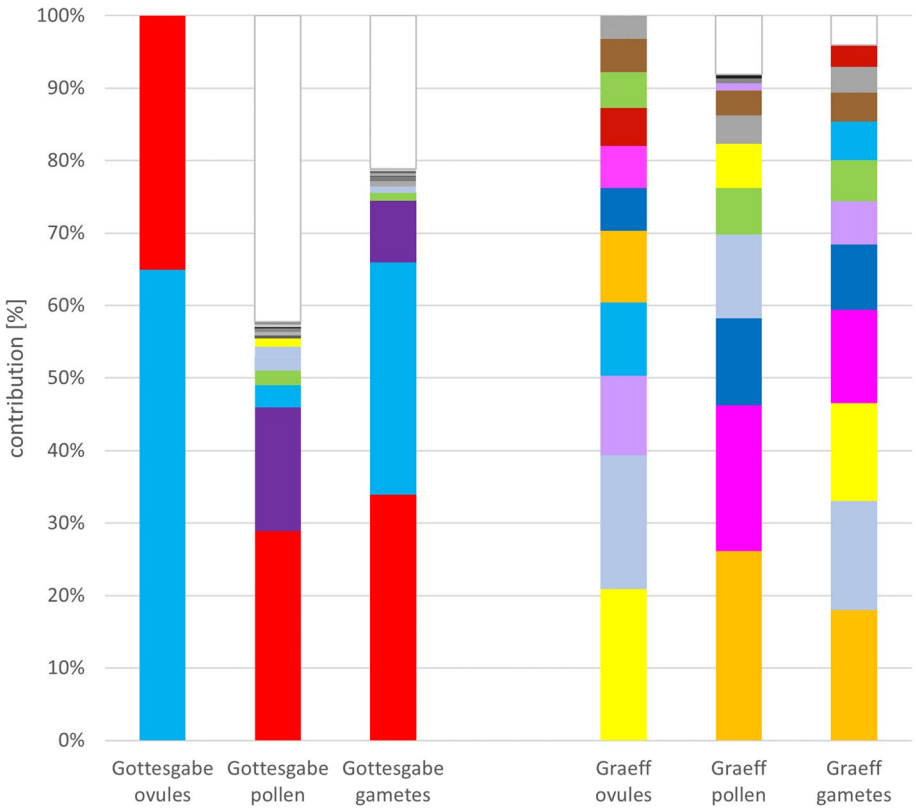

Fig. 4 Graphic display of the share of the individual parent clones in ovule, pollen and total gamete production. Pollen/gametes from unknown pollen donors/from outside of the stand are shown in white (for "Gottesgabe": including potentially unknown clones from within the stand). Clones with a share of > 1% have been coloured according to clone colours in Figs. 2 and 3. Identical clone colours in "Gottesgabe" and "Graeff" do not indicate identical genotypes

contribution shows a strongly uneven distribution of the contributions of the individual parent clones to the progeny. This result is, of course, heavily biased by the harvest of only two mother clones, which will therefore account for 100% of the ovules. However, if only the paternal/pollen contribution of the different clones is considered—which should at least partly compensate for the harvest bias—the contribution of the different adult clones to the progeny is still strongly uneven, due to the high amount of selfing (clone B) and pollination by neighbouring clones (e.g. clone A pollinated by clone C). Moreover, the relatively large proportion of pollination from outside of the stand or other unknown pollen donors can be seen.

For the seed orchard "Graeff", the diagram shows a more balanced (although not completely even) participation of the individual clones in ovule, pollen and gamete production. This pattern of clone participation is of course influenced by the more balanced harvesting of different mother clones and the more diverse pollination pattern which is promoted by the randomised "intermingled" design of the seed orchard. The proportion of pollination from unknown pollen donors from outside of the seed orchard is much lower than in "Gottesgabe".

What influence do the very uneven participation of the adult clones in the production of the progeny in "Gottesgabe", and the more balanced (although still not even) clone participation in the seed orchard "Graeff", have on the inheritance of the markers and the genetic composition and genetic diversity of the progeny? And can the potential differences within the genetic composition and diversity of parent and offspring generations be made visible by determining the classic population genetic parameters?

We checked for signs of linkage disequilibrium between all combinations of the analysed markers. The results of the pairwise analysis of linkage disequilibrium are given in Table 3 (Appendix). In both analysed progenies, all (66 of 66 possible combinations in "Graeff") or almost all (63 of 66 possible combinations in "Gottesgabe") of the possible marker combinations showed a significant deviation from the expected random distribution. In both cases, this can be explained by the limited number of parent combinations and

their different contributions to the production of the offspring (see mating patterns in Figs. 5 and 6 in the Appendix), leading to certain alleles being inherited together with above-average frequency.

Table 2 lists the population parameters determined for the progenies of "Gottesgabe" and "Graeff". Figure 7 (Appendix) shows boxplots of the population genetic parameters.

The strongly unequal participation of the different parent clones in the production of the progeny in "Gottesgabe" is reflected in the lower effective population size compared to the seed orchard "Graeff".

Interestingly, the standard population genetic parameters (calculated as mean values across the markers) do not reflect the unequal participation of the parents in the production of the progeny through a significant decrease in the genetic diversity parameters. Despite the very unequal parental contributions in "Gottesgabe", the mean number of alleles in the offspring is significantly increased compared to the parental generation ($p=0.0298$), which can probably be explained by the introduction of foreign alleles by pollination from unknown pollen donors. With about 34% pollination from unknown pollen donors played a significant role in "Gottesgabe". In the seed orchard "Graeff", the mean number of alleles in the progeny is also higher than in the parent generation, but to a lesser extent that does not reach statistical significance ($p=0.4616$). This is probably due to the much lower import of new alleles through pollination from unknown pollen donors from outside of the seed orchard (8.1%). The potential introduction of foreign genetic material through a high proportion of unknown pollen donors in "Gottesgabe" is also reflected in a higher genetic distance between parent and offspring generation compared to the seed orchard "Graeff", while in "Graeff" the lower proportion of unknown pollen donors and the more even parental contributions led to a low genetic distance between the different generations.

Other parameters like the effective number of alleles and the observed and expected heterozygosity do not show significant changes between parental and offspring generation neither in "Gottesgabe" nor in "Graeff". However, a difference can be seen in the distribution of alleles within the progeny of "Gottesgabe". The Evenness (E), a parameter that measures the similarity of the observed distribution of allele frequencies to an equal distribution of alleles (Gregorius 1990), is significantly lower in the progeny of "Gottesgabe", not only than in the parent generation ($p=4.8682\times10^{-6}$) but also in comparison to the progeny of the seed orchard "Graeff" ($p=0.0012$). The different alleles of the progeny in "Gottesgabe" are therefore more unevenly distributed than in the parent generation, while in "Graeff" the Evenness of the progeny decreases only slightly and not significantly ($p=0.1744$) compared to the parent generation.

4 Discussion

4.1 Clonal structure within the stand "Gottesgabe" and its influence on pollination patterns

Within the stand "Gottesgabe", we observed an intensive clonal structure with a "patchy" clonal architecture, i.e. with high numbers of ramets of the same clone dominating distinct areas of the stand. Such a clonal structure seems to be quite usual for natural or artificially established but then "left-alone" populations of black locust. Chang et al. (1998) analysed two stands of black locust (mixed with several oak species) in North Carolina (USA) and found clonal growth in areas from 50 up to 13,200 m^2. Only 13–14 clones were identified in both stands, which were approximately 350×225 m in size. Most clones were distributed in distinct areas. Jung et al. (2009) analysed the growth of black locust in a *Pinus thunbergia* windbreak in Japan and found extensive clonal structures. A sampling rectangle of 40×110 m contained only three different clones with over 200 ramets each and growing within relatively distinct areas. Kurokochi et al. (2010)

Table 2 Population genetic parameters for parents (P) and progenies (F1) of the stand "Gottesgabe" and the seed orchard "Graeff"

Population	Number of clones/ individuals	Mean number of alleles	Effective number of alleles	Observed heterozygosity	Expected heterozygosity	Allelic Richness	Evenness (Alleles)	Effective population size (random/ non-random)	Genetic distance (Nei)
		A	Ae	Ho	He	Ar	E	Ne	D
Gottesgabe_P	32	5.917	3.808	0.686	0.698	59.2	0.681	4	0.138
Gottesgabe_F1	433	7.917	3.196	0.645	0.654	53.2	0.481		
Graeff_P	14	7.000	4.614	0.720	0.739	72.6	0.686	9	0.03
Graeff_F1	581	7.750	4.552	0.722	0.728	69.5	0.631		

analysed the regeneration processes of six riparian black locust sites after clear-cutting and found high proportions of clonal individuals, with ramets of the same clone growing in clusters. Liesebach (2012) analysed 9 different German black locust stands of different ages and stand history. Younger stands—originally established from seedlings from a mixed seed lot—showed the first signs of beginning asexual reproduction, while older stands already formed extensive clonal structures. When working with uncharacterized (older) stands of black locust, it must therefore always be taken into account that clonal structures may have formed.

In general, it can be assumed that clonal growth influences sexual reproduction (reviewed in Charpentier (2001)). For self-compatible species, clonal growth tends to increase selfing rates. This holds true especially for populations with a "patchy" clonal architecture. Within these populations, the surrounding area of any given ramet is with a high probability dominated by other ramets of the same genotype. For wind-pollinated plants, this influences the composition of the local pollen cloud. For insect-pollinated plants like black locust, the limited radius of pollinator movement (see, e.g. Fortuna et al. (2008) and Oddou-Muratorio et al. (2005) for examples from other tree species) leads to a higher amount of pollination between ramets of the same genotype, which equals selfing in the genetic sense.

In the stand "Gottesgabe", selfing has been observed for both mother clones analysed, which shows that genetic selfing is generally possible. However, selfing rates between the two mother clones A and B differ considerably. This difference cannot be explained with stand structure, since the analysed ramets of both clones grow in comparable surroundings dominated by ramets of their own clone and few other neighbouring clones. However, for black locust, certain traits functioning to reduce selfing have been described. Surles et al. (1990) describe the flowers of black locust as protogynous, meaning that the female reproductive organs (carpels) reach maturity before the male organs (anthers). This together with the physical separation of stigmatal and antheral surfaces (Surles et al. 1990; Illies 2020) serves to reduce selfing. Inbreeding depression during seed maturation and seedling emergence (Yuan et al. 2013, 2014) further reduces the selfing rate. Different expression levels of these traits in the two mother clones analysed—together with potentially unknown genetic incompatibility mechanisms—could have played a role in influencing the selfing rates.

The clonal growth pattern does not only influence the probability of selfing. The diversity of available pollen for outcrossing is also heavily influenced. Within the surrounding area of any given ramet, other trees—next to ramets of the same clone—with a high probability represent ramets of only a few genotypes dominating the neighbouring clone patches. This effect can be seen for the offspring of mother clone A, which is mainly fathered by the neighbouring clone C. This holds true especially for the offspring in the more south-eastern parts of the clone A patch, located in closer proximity to the clone C patch. The clonal growth pattern thus promotes the forming of large full-sib families within the progenies.

A further aspect that must be taken into account is that not all clones may flower synchronously or with the same intensity. This could enhance the effects described above. A certain clone may be surrounded by neighbouring clone patches which are not flowering, flowering with low intensity or with a non-synchronous flowering time. As a consequence, even fewer pollen from synchronous-flowering clones may be available for outcrossing, which could further promote the formation of large full-sib families or increase the selfing rate. Non-synchronous or low-intensity flowering could also explain why—for example—clone B was almost not pollinated by neighbouring clone D (0.7%), but has a high selfing rate.

4.2 Uneven parental contributions and implications for the genetic composition and diversity of the progeny

If we assume that the clonal architecture of black locust stands promotes selfing and/or the forming of large full-sib families within the progeny, the dominance of a few parent trees in the production of the progeny (and thus relatively low effective population sizes) is highly likely, especially when only a few or genetically identical seed trees are harvested for seed production. One might expect this reduced genetic intermixture to have negative effects on the genetic diversity of the progeny.

However, in the stand "Gottesgabe", the introduction of foreign alleles through pollination from unknown pollen donors prevented a decrease in the mean number of alleles in the progeny. This high amount of pollination from unknown pollen donors can be explained by the relatively high number of black locust trees growing within the neighbourhood of the analysed stand.

Nevertheless, due to the unequal parental contributions and high selfing rates, a more uneven allele distribution was observed within the progeny of "Gottesgabe". Such a relatively uneven allele distribution in, e.g. harvested seed, can lead to an unintended reduction in genetic diversity in randomly selected subsets and thus to a reduction in the number of alleles in progeny derived from the seed. Moreover, it has to be considered that a compensation for the risk of losing genetic diversity through pollination from outside may not always be given. Black locust, as a non-native species in Europe, may grow in quite isolated locations,

where pollen from neighbouring populations is not available in large amounts. This is particularly true as black locust is an insect-pollinated species and the flight range of pollinating insects is a limiting factor for the achievable pollination distances. Most pollination events in insect-pollinated species take place in relative proximity to the pollinated tree (Oddou-Muratorio et al. 2005; Fortuna et al. 2008). It must also be taken into account that pollination from outside may not always be desirable. When harvesting from a selected seed stand, one can imply that the selection of this seed stand was based on desirable phenotypic characteristics. Tree selection and breeding in black locust is mostly focused on the improvement of stem straightness and the level of biomass production (reviewed in Ábri et al. (2023)). Pollination from outside might introduce inferior genetic material.

4.3 Seed orchards as the better alternative?

Seed orchards usually consist of selected clones with favourable phenotypic traits. Several ramets of these clones are arranged in a randomised design to ensure a diverse pollination pattern and thus a good genetic intermixture based on a high number of different parent combinations. This aims to ensure the preservation of favourable genetic traits in the next generation while at the same time maintaining genetic diversity (reviewed in Funda and El-Kassaby (2012)).

The seed orchard "Graeff" deviated in part from an ideal seed orchard design. Due to the unplanned multiple selection of actually genetically identical genotypes, the seed orchard contained fewer clones than intended and the number of ramets per clone was highly variable. Such a variation in ramet number per clone can promote an unequal participation of the individual parent clones in the production of the progeny, which hinders the free intermixture of genetic material in the offspring. Moreover, some clones of the seed orchard did not flower (yet), further reducing the number of different clones actively involved in reproduction, although this disadvantage may disappear in the future as the clones get older.

Nevertheless, in the progeny of the seed orchard "Graeff" genetic intermixture was more diverse than in "Gottesgabe", due to a less unequal participation of the parent clones and a lower selfing rate. The allele numbers and an even allele distribution were maintained in combination with a lower rate of (potentially unwanted) pollination from outside. Optimised seed orchards with a higher number of (flowering) clones in more equal ramet numbers, could possibly ensure an even better genetic intermixture.

5 Conclusions

The comparison we have made between a clonal stand and a seed orchard of black locust is only a single example. Further investigations would certainly be desirable. Nevertheless, certain conclusions can be drawn. Clonal growth in black locust (and potentially other clonal species) can lead to large areas of a stand being dominated by only a few or even a single clone. If the genetic composition of a stand is unknown, this might lead to the unintended seed harvest from multiple trees of the same genotype, especially if seeds are harvested from trees located in relatively close proximity to each other. When harvesting seed material from clonal stands, it would therefore be advisable to—as already suggested by Schirmer and Cremer (2020)—either analyse the genetic structure of the stand or at least harvest from trees located as far apart from each other and as well dispersed over the whole stand as possible. However, the former is associated with relatively high costs and time expenditure and the latter cannot always be guaranteed since the accessibility of the stand could be impeded by the abundant formation of further young root suckers.

Next to the creation of an unintended harvest bias, "patchy" clonal growth will also influence the genetic intermixture in the harvested seed lot by promoting higher selfing rates and/or the formation of large full-sib families due to the above-average mating frequency of neighbouring clone patches. All this might negatively influence the level of genetic diversity, especially if pollination from outside of the stand is low.

For plants with "patchy" clonal growth, seed orchards have the advantage that the randomised planting scheme ensures better spatial intermixture of the different clones. This promotes a higher number of different parent combinations involved in the production of the progeny. Trees in the seed orchards are normally easily accessible for harvesting. This, together with the documentation of the clone positions within the planting scheme facilitates the harvesting of genetically different clones, leading to a more genetically mixed and diverse seed lot.

However, when establishing seed orchards, the effort should be made—as already suggested by Naujoks et al. (2012) and Schirmer and Cremer (2020)—to genetically check selected clones in order to prevent unwanted multiple selections of actually identical clones, which can lead to lower clone numbers and unequal ramet numbers per clone and would reduce some of the benefits described above.

Taken together, the establishment and maintenance of a seed orchard costs time and money and will certainly be subject to economic considerations. However, from a genetic point of view, in order to produce high-quality forest reproductive material from clonally growing species, seed production in seed orchards is certainly preferable to harvesting in uncharacterized clonal stands.

Appendix

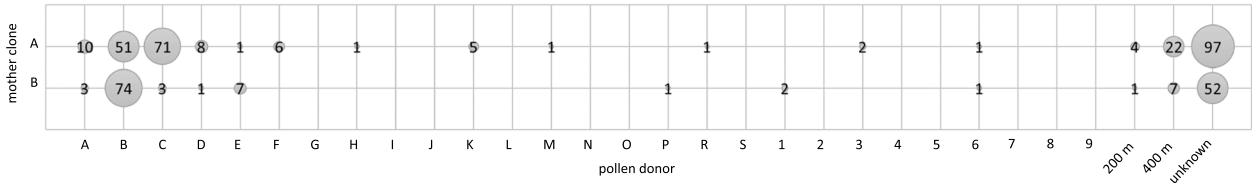

Fig. 5 Mating scheme of the analysed progeny of the stand "Gottesgabe". Bubble size depends on the number of offspring individuals of the respective parent combination

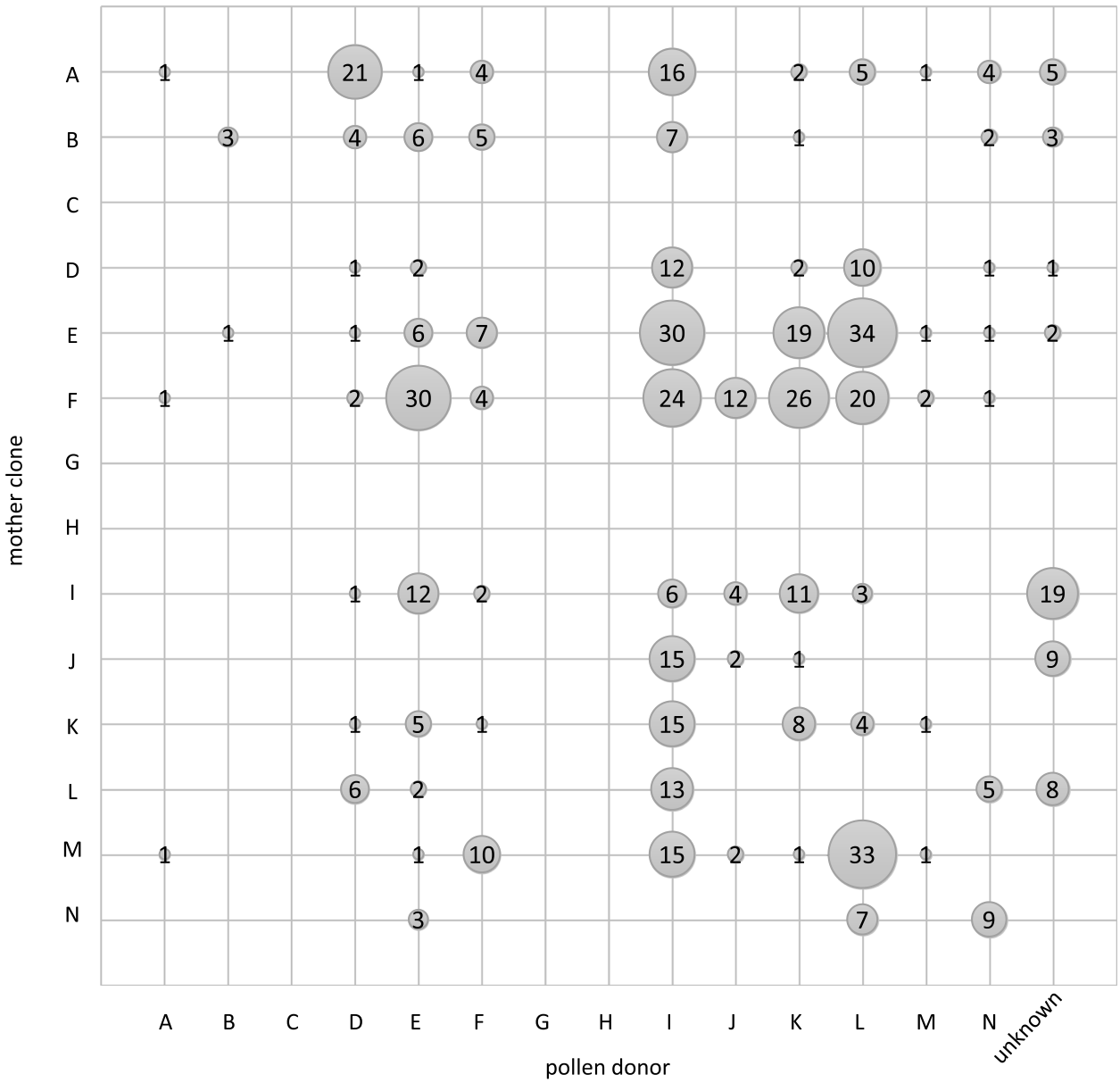

Fig. 6 Mating scheme of the analysed progeny of the seed orchard "Graeff". Bubble size depends on the number of offspring individuals of the respective parent combination

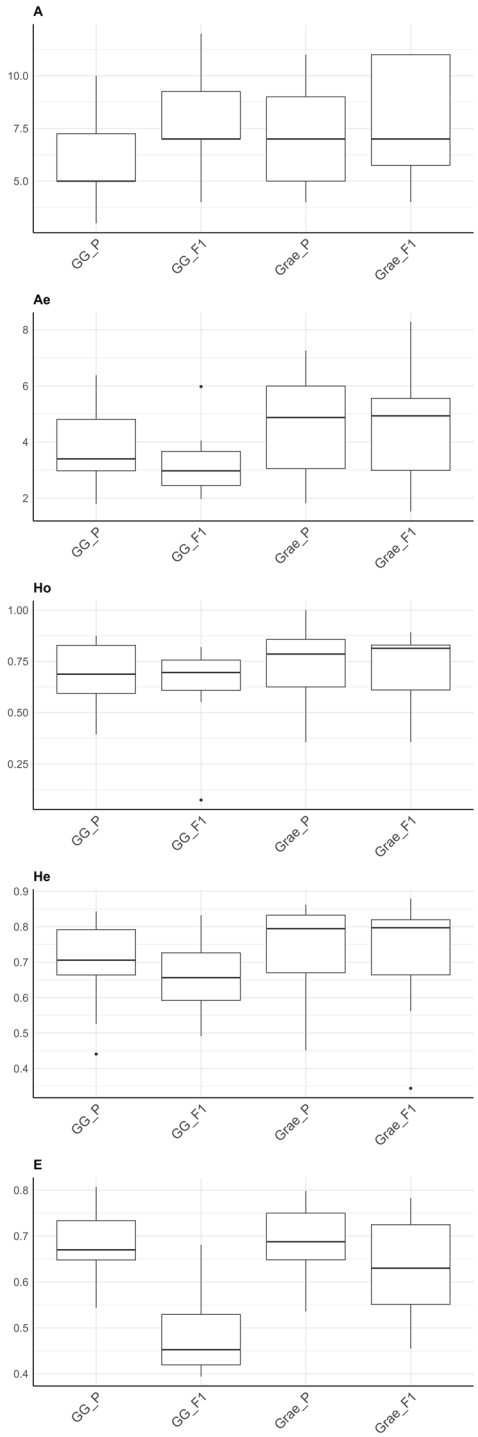

Fig. 7 Boxplots of the population genetic parameters: A: mean number of alleles, Ae: mean effective number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, E: evenness, GG_P: "Gottesgabe" parent generation, GG_F1: "Gottesgabe" offspring generation, Grae_P: "Graeff" parent generation, Grae_F1: "Graeff" offspring generation

Table 3 Results of the pairwise analysis of linkage disequilibrium in parent and offspring generation of "Gottesgabe" and "Graeff". GG_F1: "Gottesgabe" offspring generation, GG_P: "Gottesgabe" parent generation, Grae_F1: "Graeff" offspring generation, Grae_P: "Graeff" parent generation, S.E.: standard error, Switches: number of recombinations between the alleles of the two loci

Pop	Locus 1	Locus 2	*P*-value	S.E	Switches
GG_F1	R05	R06	0	0	8656
GG_F1	R05	R_106	0	0	15,565
GG_F1	R06	R_106	0	0	14,231
GG_F1	R05	R01B	0	0	7949
GG_F1	R06	R01B	0	0	7307
GG_F1	R_106	R01B	0	0	17,297
GG_F1	R05	R08	0	0	6766
GG_F1	R06	R08	0	0	6266
GG_F1	R_106	R08	0	0	14,719
GG_F1	R01B	R08	0	0	7953
GG_F1	R05	R16	0	0	6817
GG_F1	R06	R16	0	0	6433
GG_F1	R_106	R16	0	0	13,941
GG_F1	R01B	R16	0	0	6668
GG_F1	R08	R16	0	0	5900
GG_F1	R05	R035	0	0	7882
GG_F1	R06	R035	0	0	6497
GG_F1	R08	R035	0	0	6326
GG_F1	R16	R035	0	0	6417
GG_F1	R06	R032	0	0	8444
GG_F1	R_106	R032	0	0	31,691
GG_F1	R01B	R032	0	0	8418
GG_F1	R08	R032	0	0	8531
GG_F1	R16	R032	0	0	6546
GG_F1	R05	R02	0	0	6132
GG_F1	R06	R02	0	0	5894
GG_F1	R_106	R02	0	0	14,223
GG_F1	R01B	R02	0	0	5992
GG_F1	R08	R02	0	0	5606
GG_F1	R16	R02	0	0	5106
GG_F1	R035	R02	0	0	5890
GG_F1	R032	R02	0	0	5865
GG_F1	R05	R109	0	0	10,944
GG_F1	R06	R109	0	0	9103
GG_F1	R08	R109	0	0	8726
GG_F1	R16	R109	0	0	8876
GG_F1	R035	R109	0	0	8996
GG_F1	R032	R109	0	0	9738
GG_F1	R02	R109	0	0	8207
GG_F1	R05	R200	0	0	6867
GG_F1	R06	R200	0	0	6351
GG_F1	R_106	R200	0	0	13,119
GG_F1	R01B	R200	0	0	6564

Pop	Locus 1	Locus 2	*P*-value	S.E	Switches
GG_F1	R08	R200	0	0	6019
GG_F1	R16	R200	0	0	5736
GG_F1	R035	R200	0	0	6197
GG_F1	R032	R200	0	0	6161
GG_F1	R02	R200	0	0	5382
GG_F1	R109	R200	0	0	8797
GG_F1	R05	R206	0	0	13,105
GG_F1	R06	R206	0	0	11,669
GG_F1	R_106	R206	0	0	22,273
GG_F1	R01B	R206	0	0	13,231
GG_F1	R08	R206	0	0	10,945
GG_F1	R16	R206	0	0	11,884
GG_F1	R035	R206	0	0	10,747
GG_F1	R032	R206	0	0	17,318
GG_F1	R02	R206	0	0	11,303
GG_F1	R109	R206	0	0	16,593
GG_F1	R200	R206	0	0	11,440
GG_F1	R05	R032	4E−05	0	8431
GG_F1	R035	R032	0.0001	0	8147
GG_F1	R01B	R109	0.0069	0	9304
GG_F1	R_106	R035	0.096	0.01	22,655
GG_F1	R_106	R109	0.2215	0.02	17,288
GG_F1	R01B	R035	0.2391	0.03	13,988
GG_P	R06	R032	0	0	4489
GG_P	R16	R206	0.0077	0	2476
GG_P	R035	R206	0.0125	0	5843
GG_P	R06	R109	0.0152	0.01	4590
GG_P	R08	R109	0.0243	0	9805
GG_P	R16	R032	0.0321	0.01	2293
GG_P	R16	R109	0.0401	0.01	3731
GG_P	R05	R035	0.0405	0.01	2500
GG_P	R032	R109	0.0427	0.01	5608
GG_P	R08	R206	0.0535	0.01	8499
GG_P	R01B	R035	0.0537	0.01	3394
GG_P	R01B	R032	0.0549	0.01	2660
GG_P	R08	R035	0.067	0.01	9746
GG_P	R035	R02	0.1059	0.02	2655
GG_P	R109	R200	0.1188	0.02	4926
GG_P	R_106	R206	0.1321	0.01	13,496
GG_P	R05	R206	0.1357	0.02	2118
GG_P	R06	R01B	0.1752	0.03	1921
GG_P	R16	R200	0.1757	0.03	1818
GG_P	R035	R200	0.1772	0.02	4303
GG_P	R16	R035	0.1801	0.02	2924
GG_P	R_106	R200	0.188	0.01	10,930
GG_P	R06	R16	0.1945	0.03	1659
GG_P	R_106	R01B	0.2001	0.02	9473
GG_P	R08	R02	0.2002	0.02	4308
GG_P	R08	R032	0.2338	0.02	7477
GG_P	R16	R02	0.2659	0.04	973
GG_P	R08	R16	0.2685	0.02	4644
GG_P	R05	R109	0.3038	0.03	3135
GG_P	R032	R200	0.3316	0.03	4065
GG_P	R05	R_106	0.3608	0.02	8070
GG_P	R032	R206	0.3655	0.02	4542
GG_P	R01B	R109	0.3787	0.02	4076
GG_P	R02	R200	0.3921	0.04	1578
GG_P	R109	R206	0.4304	0.02	6308
GG_P	R_106	R02	0.4324	0.02	8583
GG_P	R05	R200	0.5084	0.04	1472
GG_P	R_106	R032	0.5157	0.01	12,031
GG_P	R06	R206	0.5181	0.03	3503
GG_P	R01B	R206	0.5471	0.03	2954
GG_P	R200	R206	0.5603	0.03	3834
GG_P	R_106	R035	0.5677	0.01	15,099
GG_P	R035	R109	0.6039	0.02	7208
GG_P	R05	R16	0.6142	0.04	928
GG_P	R06	R200	0.6301	0.03	2654
GG_P	R06	R_106	0.6473	0.02	10,443
GG_P	R_106	R109	0.6475	0.01	14,591
GG_P	R_106	R16	0.6514	0.02	8793
GG_P	R05	R08	0.6522	0.03	3816
GG_P	R05	R032	0.6934	0.03	2130
GG_P	R01B	R200	0.6994	0.03	2097
GG_P	R01B	R02	0.714	0.03	1182
GG_P	R06	R035	0.7331	0.02	4052
GG_P	R02	R109	0.7588	0.02	3454
GG_P	R01B	R08	0.7736	0.02	5324
GG_P	R06	R08	0.8142	0.02	6087
GG_P	R05	R06	0.8284	0.02	1491
GG_P	R06	R02	0.8296	0.03	1573
GG_P	R_106	R08	0.8383	0.01	19,283
GG_P	R05	R01B	0.8474	0.03	1073
GG_P	R01B	R16	0.8519	0.03	1339
GG_P	R035	R032	0.9245	0.01	5129
GG_P	R032	R02	0.9412	0.01	2367
GG_P	R08	R200	0.9527	0.01	6273
GG_P	R02	R206	0.9558	0.01	2386
GG_P	R05	R02	1	0	756
Grae_F1	R05	R06	0	0	8079
Grae_F1	R05	R_106	0	0	7095
Grae_F1	R06	R_106	0	0	14,392
Grae_F1	R05	R01B	0	0	3071
Grae_F1	R06	R01B	0	0	10,974
Grae_F1	R_106	R01B	0	0	9089
Grae_F1	R05	R08	0	0	6859
Grae_F1	R06	R08	0	0	17,917
Grae_F1	R_106	R08	0	0	13,881
Grae_F1	R01B	R08	0	0	9717
Grae_F1	R05	R16	0	0	1976
Grae_F1	R06	R16	0	0	8781
Grae_F1	R_106	R16	0	0	7621

Pop	Locus 1	Locus 2	*P*-value	S.E	Switches
Grae_F1	R01B	R16	0	0	3097
Grae_F1	R08	R16	0	0	7615
Grae_F1	R05	R035	0	0	6644
Grae_F1	R06	R035	0	0	17,294
Grae_F1	R_106	R035	0	0	13,331
Grae_F1	R01B	R035	0	0	9541
Grae_F1	R08	R035	0	0	15,850
Grae_F1	R16	R035	0	0	7254
Grae_F1	R05	R032	0	0	4604
Grae_F1	R06	R032	0	0	13,673
Grae_F1	R_106	R032	0	0	14,823
Grae_F1	R01B	R032	0	0	5660
Grae_F1	R08	R032	0	0	11,085
Grae_F1	R16	R032	0	0	4519
Grae_F1	R035	R032	0	0	11,750
Grae_F1	R05	R02	0	0	1834
Grae_F1	R06	R02	0	0	6470
Grae_F1	R_106	R02	0	0	5925
Grae_F1	R01B	R02	0	0	2555
Grae_F1	R08	R02	0	0	5546
Grae_F1	R16	R02	0	0	1651
Grae_F1	R035	R02	0	0	5209
Grae_F1	R032	R02	0	0	4338
Grae_F1	R05	R109	0	0	5360
Grae_F1	R06	R109	0	0	16,734
Grae_F1	R_106	R109	0	0	13,094
Grae_F1	R01B	R109	0	0	7735
Grae_F1	R08	R109	0	0	14,656
Grae_F1	R16	R109	0	0	5665
Grae_F1	R035	R109	0	0	14,385
Grae_F1	R032	R109	0	0	10,491
Grae_F1	R02	R109	0	0	4289
Grae_F1	R05	R200	0	0	2218
Grae_F1	R06	R200	0	0	7954
Grae_F1	R_106	R200	0	0	7059
Grae_F1	R01B	R200	0	0	3174
Grae_F1	R08	R200	0	0	6968
Grae_F1	R16	R200	0	0	2264
Grae_F1	R035	R200	0	0	6544
Grae_F1	R032	R200	0	0	5044
Grae_F1	R02	R200	0	0	1789
Grae_F1	R109	R200	0	0	5462
Grae_F1	R05	R206	0	0	7308
Grae_F1	R06	R206	0	0	16,519
Grae_F1	R_106	R206	0	0	15,147
Grae_F1	R01B	R206	0	0	10,088
Grae_F1	R08	R206	0	0	16,223
Grae_F1	R16	R206	0	0	8664
Grae_F1	R035	R206	0	0	16,124
Grae_F1	R032	R206	0	0	17,271
Grae_F1	R02	R206	0	0	6451
Grae_F1	R109	R206	0	0	15,931
Grae_F1	R200	R206	0	0	6771
Grae_P	R_106	R206	0.0376	0	9702
Grae_P	R16	R035	0.1342	0.03	1251
Grae_P	R06	R032	0.1475	0.01	5310
Grae_P	R01B	R08	0.1899	0.02	2005
Grae_P	R035	R109	0.2001	0.03	1914
Grae_P	R06	R200	0.2039	0.02	4180
Grae_P	R035	R206	0.2216	0.02	2351
Grae_P	R16	R109	0.2638	0.03	1851
Grae_P	R05	R_106	0.2796	0.02	6317
Grae_P	R032	R206	0.2851	0.02	3558
Grae_P	R109	R200	0.3176	0.03	2244
Grae_P	R16	R032	0.4221	0.03	2202
Grae_P	R16	R206	0.4297	0.03	2263
Grae_P	R_106	R08	0.4779	0.01	9662
Grae_P	R06	R02	0.4946	0.02	3639
Grae_P	R06	R206	0.5241	0.02	5247
Grae_P	R06	R_106	0.5294	0.01	12,108
Grae_P	R08	R109	0.5426	0.03	3197
Grae_P	R06	R035	0.5481	0.02	3677
Grae_P	R_106	R032	0.5601	0.01	9643
Grae_P	R06	R16	0.6021	0.02	3634
Grae_P	R08	R032	0.7349	0.02	3524
Grae_P	R_106	R16	0.8028	0.01	7310
Grae_P	R_106	R200	0.8535	0.01	8054
Grae_P	R_106	R109	0.9052	0.01	9174
Grae_P	R06	R08	0.9312	0.01	5225
Grae_P	R05	R06	1	0	2937
Grae_P	R05	R01B	1	0	851
Grae_P	R06	R01B	1	0	3286
Grae_P	R_106	R01B	1	0	6680
Grae_P	R05	R08	1	0	1807
Grae_P	R05	R16	1	0	982
Grae_P	R01B	R16	1	0	1144
Grae_P	R08	R16	1	0	2209
Grae_P	R05	R035	1	0	1050
Grae_P	R_106	R035	1	0	7492
Grae_P	R01B	R035	1	0	1171
Grae_P	R08	R035	1	0	2363
Grae_P	R05	R032	1	0	1799
Grae_P	R01B	R032	1	0	1996
Grae_P	R035	R032	1	0	2456
Grae_P	R05	R02	1	0	1029
Grae_P	R_106	R02	1	0	7367
Grae_P	R01B	R02	1	0	1146
Grae_P	R08	R02	1	0	2312
Grae_P	R16	R02	1	0	1358
Grae_P	R035	R02	1	0	1382
Grae_P	R032	R02	1	0	2280
Grae_P	R05	R109	1	0	1483

Pop	Locus 1	Locus 2	*P*-value	S.E	Switches
Grae_P	R06	R109	1	0	4692
Grae_P	R01B	R109	1	0	1718
Grae_P	R032	R109	1	0	3199
Grae_P	R02	R109	1	0	1942
Grae_P	R05	R200	1	0	1138
Grae_P	R01B	R200	1	0	1441
Grae_P	R08	R200	1	0	2586
Grae_P	R16	R200	1	0	1586
Grae_P	R035	R200	1	0	1587
Grae_P	R032	R200	1	0	2708
Grae_P	R02	R200	1	0	1562
Grae_P	R05	R206	1	0	1805
Grae_P	R01B	R206	1	0	1989
Grae_P	R08	R206	1	0	3542
Grae_P	R02	R206	1	0	2328
Grae_P	R109	R206	1	0	3185
Grae_P	R200	R206	1	0	2622

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Authors' contributions
Conceptualisation: Heike Liesebach, Volker Schneck; Methodology: Heike Liesebach, Birte Pakull; Formal analysis and investigation: Birte Pakull, Heike Liesebach; Writing – original draft preparation: Birte Pakull; Writing – review and editing: Heike Liesebach, Volker Schneck; Funding acquisition: Heike Liesebach.

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Availability of data and materials
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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors gave their informed consent to this publication and its content.

Competing interests
The authors declare that they have no competing interests.

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