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The picture illustrates polycross with selection forwards. A good looking tree in a good progeny has been identified. The author collects a needle sample from which DNA will be extracted and the pollen parent identified, this information will be used for the final selection.

With this method crosses and field testing need to be done only once in a breeding cycle. The cost is reduced and the gain per time unit increased.

Photo: Ola Rosvall.

## Polymix breeding with selection forwards

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## Summary and overall recommendations

- A method – here called polymix breeding (polymix with parent analyses, PMX/WPA) - is discussed. Selected genotypes are pollinated with a pollen mix of selected genotypes. Later trees in field trials with the recruitment population get their father decided by markers. Selection for further breeding is partly based on pedigree as revealed by markers.
- Polymix breeding applied in an optimized way has the potential to be more powerful than current Scots pine breeding. Pollen can be resolved without being a major effort of the breeding operation provided a lab with relevant experience exists and wants to assist.
- Thus Polymix breeding is recommended to be more carefully explored by theoretical means, like POPSIM simulations and continuing the discussion initiated in this work report.
- Currently I do not recommend to mix more than about 25 genotypes in a polycross mix. Otherwise the effort for the marker analysis will raise, indecisive situations will be more common and dependence of development and competence higher.
- An alternative for a Swedish Polymix breeding for Scots pine is suggested (but I am by no way convinced it is the best). For a “breeding population” 375 genotypes are selected. The use of many pollen and several pollen mixes in the same breeding population is suggested. 25 pollen in each polymix and five pollen-mixtures (125 different pollen together) for a “breeding population”. 250 genotypes are pollinated with one of these mixtures, different for different genotypes, around 50 seed parents for each pollenmix. This recruitment population is growing in field tests, a pre-selection is done and analyzed by markers, and after that the selection to the breeding population is done as above and the steps are repeated in the next cycle.
- When the method has been successfully implemented for pine, it is suggested to consider it for spruce.
- Objects existing now where polycross crosses have been made are unlikely to be suitable objects for polymix breeding. The pollen chosen for the mix is unlikely to be suitable.
- It is suggested that Skogforsk creates objects as feasibility studies as a part of the operational breeding. Progeny-testing with pollen mix has been the main vision for Scots pine for the past 15 years, it ought to exist several objects (trees outside breeding stations, trees with accelerated breeding, top grafts, conventional grafts) where polymix crosses are planned in the near future to evaluate breeding values, which can easily be converted to polymix breeding. It seems not a good idea to do polycross crosses any more without keeping the option to convert them to polymix breeding open.

- The particular algorithms and procedures needed for optimizing polymix breeding need not be developed until a material becomes mature for decisions. It is known it will work, but the technology and strategy development will improve while the materials mature.
- Once the pollen and seed parents become available for crosses, it is perhaps more efficient for long term breeding to do many crosses with known parents (e.g. SPM or the grandparents designs described by Lindgren et al., 2008), than to use the polymix progenies for forward breeding. Thus it is currently recommended to go for polymix breeding only if the comparatively high accuracy of the breeding values of the parents is regarded valuable (e.g. for seed orchard establishment) or for gaining practical experience.

## Introduction

Conventional progeny testing with polycross gives reliable breeding value of the parents. Differences among pollen parents even out as many pollen parents are mixed. Pollen parents contribute only statistical noise and are sources of uncertainty in spite of that each zygote carries as much information about the pollen parent as about the seed parent. Selection forwards in a polycross progeny is not a good idea as the father is not known. But by using modern marker technology the father can be identified among a number of candidates. The information the pollen parent in the pollen mix carries can be used and polymix progenies can become a resource for continued breeding. Polymix with parental analyses (PMX/WPA)-breeding was suggested by Lambeth et al. (2001). The idea is to pollinate with a mixture of pollen, and identify and utilize the male parenthood afterwards. Lambeth et al. (2001) suggested 3 models, 1) that “the identification tags are thrown away” (thus the information of the mother was not kept), 2) that all progeny plants are pedigreed and 3) that only the best trees in the progenies are pedigreed. A variant of the system was used operatively by the company Weyerhaeuser, where Lambeth was employed. It is not known to have been used by other breeding organizations. Neither is little published or attention paid, e.g. as proceeding articles.

## Molecular markers technology

### ARE MOLECULAR MARKERS POWERFUL ENOUGH?

Molecular markers have to be powerful enough to resolve parenthood among candidates. The actual resolution power in experiments is often lower than expectations raised by proponents of their use (cf Lambeth et al 2001, Wheeler et al 2006). Practical complications (like nul-alleles, badly working samples, DNA contamination, too much or too little DNA, low quality DNA, failed efforts to make methodological short-cuts, insufficient polymorphism, insufficiently developed systems) may make the marker analysis more resource demanding and more depending on established competence than initially imagined. It may happen, as has happened so often before, that the molecular option promises more than it could keep up to. The parental analyses may become more complicated as breeding advance over generations, as relatives will become more common and relatives share markers at different degrees. However, recent results with parentage identification with Scots pine

(Wang et al., 2009) are promising and prove that it is realistic with modern marker techniques, at least for some cases in Scots pine. The skill needed to do this for Scots pine in Sweden exists at the moment. I believe that the methods of today can identify 98% among 40 candidate fathers correctly. In theory the accuracy is higher, but there are other sources of uncertainty than the evident. If the number of candidates (pollen parents in the mix) is above 25 the analyses becomes seems more expensive and complicated (more systems than the most suitable of those already developed will be needed).

## **ARE MOLECULAR MARKERS TOO EXPENSIVE?**

Wang et al. (2009) estimated that 2000 trees can be analyzed in a man-year. With overheads, laboratories, material and supervision this adds 50€ per tree. In addition to that it is an initiation cost of say 10 000€ should be considered. This requires that the analysis systems are developed for the specific species and that competence and experience and laboratories and skill for the statistical analyses exist. The added costs will be considerable, if an infrastructure does not exist. There are advantages of scale and molecular analyses develop over time, analyses will become more automat zed and costs will decrease, even if the magnitude is uncertain. The overall cost decrease has not been that dramatic as the cost reduction of some steps have been (e.g. DNA extraction and gel reading remain time consuming after decades of development). The development and rationalization is certainly at the advantage for lab activities compared to field activities, which makes designs like this more competitive over time, but still the speed at which the transition is occurring is often overestimated by enthusiastic proponents.

Laboratory cost is not the total cost and it is probably not the dominating cost in a feasible program. There are also costs for sampling, documentation, supervision, field experiments, measurement, compute ring, analyses, decision making, reporting, crosses, pollen management, seed management, clone archives, breeding archive etc. depending on the system applied. Cost considerations in Wang et al. (2009) indicated that the molecular marker costs are not the main costs of a tree breeding program using markers.

## **Suggestion of a reference alternative for Polymix breeding applicable to Swedish scots pine**

Polymix breeding can be made in many ways. Here I focus on a reference alternative, but also discusses many others the alternatives. The reference alternative need not be optimal and should be seen as a starting point for making designs for real application, not an exact recommendation. The reference starts with a situation where a material is available comprising of double pair matings from 50 plus trees available for phenotypic selections in field trials.

The reference alternative is that 375 phenotypes with known parents have been selected as candidates in field trials. From those 250 are used as females and 125 as males. Pollen mixes are made in batches of 25 and the females are structured in compartments of 50, which are pollinated with different pollen mixes. Field trials are established and constitute a recruitment population. The mothers are known. Based on the performance of the known mothers 700 good phenotypes are selected as candidates and subject to marker analyses. Based on the added

information from the marker analyses 375 are selected and a new cycle can begin. This selection is made considering the constraints on the reduction in diversity per generation applied in the Swedish breeding.

## **THE POTENTIAL OF THE POLYMIX BREEDING OPTION**

Instead of two rounds of crosses as in the Swedish Scots pine baseline strategy, in this polycross strategy uses only one round, serving both the functions of testing and creating a recruitment population for selection forwards at the same time. This causes conception difficulties. A well defined breeding population does not exist any more. Which of the parents that transfer their genes to the next breeding generation will not be decided when the crosses are made, but to some extent when selection is done in the following generations. There will be relatives in the pedigree of mates. However, there are many options to handle this but how to do this is neither evident nor easy. The concept family becomes unclear when there are overlapping male and female families. The “breeding population” becomes many times larger than some are accustomed to, even if it “actually” is not larger looking retrospectively. A change in way of considering breeding generation number is also needed, as breeding will not be synchronized in the advanced generations in the future. Limits on the increase of group coancestry both per year and per generation in the breeding stock could be applied rather than a general rule assuring balance.

## **SHOULD THE SAME PARENTS BE USED BOTH AS MALE AND FEMALES?**

There may be sources of male parents which are hardly available as female parents, e.g. trees in field trials may be selected and harvested for pollen, while they are hardly available as female mating partners. However pines must be rather mature to get sufficient pollen, and when it may not be conveniently available and the time window for field collection is narrow in a busy period, so this will be a real option only in some rare special cases.

Female flowers and male flowers may develop at different genotypes so it is reason to use them as either females or males.

If selections are used as males in a Polymix, that pollen may be ineffective and the candidates never get any progeny because the pollen did not do it. The risk is smaller with females that they never get crossed. If it is relatively expensive to make and maintain grafts it is an argument to use them both as pollen parents and seed parents.

The grafts from which pollen is obtained need not be archived waiting for test results. That is an argument to have candidates which contribute only by pollen.

If males are also females, the males will get reliable breeding values and thus the selections may be based not only on phenotypic performance and the seed parents breeding value, but also the pollen parents breeding value.

If males and females are different, a larger candidate population can be involved at the same cost. If the paternal breeding values are not actually used for selection as a balance among pollen parents is desired it seems the information of breeding value is lost. However if the breeding value information can be used

and better pollen parents somewhat overrepresented it may be worth using pollen parents which are also tested as seed parents.

Perhaps the solution will be a mixed one mainly because of practical reasons; some candidates will only be used as pollen parents and some both as pollen parents and seed parents.

### **WHAT SHARE OF FEMALES VERSUS MALES?**

Each time a selection is done, both a father and mother is selected. If pollen parents and seed parents are different populations, problems with overrepresentation are reduced if genders are equally represented. But there could be many reasons to head for different representation. One may be cost or convenience, pollen management costs and equipment for pollen management and staff for pollen management is often rather limiting if the number of pollen is in the hundreds, while pollinating some more females may be rather simple. The availability of sexual structures may be different for the genders. To have different numbers for the reference case is mainly a question about expected selection efficiency. The seed parents are known from the identification of the recruitment population. The candidates are selected mainly from seed parents with good progeny and in the final selection after marker analyses the genders will be balanced. As the selection depending on the seed parents with progeny-test will be much more accurate than the selection depending on the father with phenotypic selection, and wherefore it better to get more seed parents to select among than pollen parents. The reference alternative suggests double as many, but the “optimal” share of pollen parents may quite well be somewhat lower.

### **SELECTION INTENSITY**

The gain comes from selection intensity of the initial selection backwards on the mothers and on the selection intensity forwards to get phenotypes. The number of pollen parents does not matter that much in the first approximation. Balance among pollen parents is a constraint and reduces the selection intensity in the selection forwards, and if there are many more pollen parents than needed, the constraint will be less and in that meaning the selection intensity will be higher. Given the number of parents, the size of the batches they are organized in matter rather little. The best fraction of a large sample will correspond to a little higher selection intensity than the same best fraction from a small sample, but the difference is small. The more candidates selected, the lower the selection intensity in that step, but the more candidates to select a certain size of the breeding population from, the higher the selection intensity will be in the final step. This is one of the considerations for optimizing the designs.

If rather few pollen parents and a low share of pollen parents are used, it can be a good idea to take better phenotypes as pollen parents than female parents, as they will get more represented in the pre-selections than the females will be.



If the pollen parents are the same as seed parents tested by poly-mixes or if pollen parents get their breeding value decided via marker identification it could be motivated to have more fathers to select among. If the minimum number of pollen parents is used it will not be any gain by selecting on the paternal side and it will be constraints lowering gain to get pollen parents equally represented.

It seems adviceable using more pollen parents than what is considered the minimum number required (say considerable more than 25 for a Swedish breeding population), because the pollen parents will be differently represented among the pre-selections and it will probably be costly in gain to force balance which still not be achieved because of poor performance of some pollen. Set 50 as a minimum number at least as long as there is little actual experience from the Swedish program.

When selections are it could be a good idea to over-represent some depending on the predicted breeding value of the parents (cf. Ruotsalainen & Lindgren, 1998).

### **WHY USE SEVERAL POLY-CROSS BATCHES?**

If there are maximum 25 pollen parents there may not be reasons strong enough to make more than one pollen batch. If there are more, it may be difficult and expensive to separate the pollen parents by markers.

Practicabilities about pollen availability may be a motive for a system with different batches and one reason for compartments of the breeding population may be availability of pollen.

### **What is the benefit of using many pollen in a polycross batch?**

The pollen parents should be separated by markers and that become more difficult and costly the more candidate pollen parents where are (see below). The chance of a mistake with a wrongly labeled pollen increases and a mistake in the analyses increases if more systems are involved. As it is desirable to get pollen from recently selected candidate trees, which has not been tested yet, the pollen amounts may be limiting and that could be a reason to use many pollen in a batch.

Even if pollen from different parents are mixed in equal quantities, they will still be differently effective in fertilization and have different representation in the progeny. Pollen differ in competitiveness. Differences in collection, extraction and storage may contribute more differentiation. The availability of pollen varies widely, in particular on young trees. If many pollen are used it matters less that their contributions are balanced, and thus the lowest pollen producer is less limiting. Many pollen give more comparable breeding value estimates among batches and less influence of dominance. The selection intensity becomes slightly higher if there are large batches, and large pollen batches makes the constraints imposed while fathers get known less limiting. If the aim is selections forwards there are reasons to have a balance between male and females among candidates, and that could sometimes be an argument for using rather many pollen.

### **What is the benefit of using few pollen parents in a batch?**

It is easier to handle few pollen in a mixture. The marker analyses will be simpler, cheaper and more reliable the fewer pollen which are mixed in a batch. The risk decreases that a mistake of identification of a pollen parent makes the mixture less valuable. The selection intensity is smaller to select the best among a small number than to select the same proportion in a larger number. The breeding values get less accurate when compared with results obtained by other pollen mixes, and because of dominance and G\*E. It can be difficult to avoid overrepresentations of a few pollen parents.

If there are relatives among the pollen-parents, it is more difficult to separate them by markers as relatives share genes. It is easier and more desirable to avoid relatedness in the early stage of a breeding program than at a more advanced stage. This is a reason to argue that the most suitable number of pollen in a polycross mix will decrease over time.

An advantage with few pollen parents is that the selection intensity when pollen parents are phenotypically selected is higher the fewer which are used. But this is more a question about selection intensity over the whole program than how pollen are distributed in batches.

### **Why pollenmixes with 25 different pollen?**

Where is the limit of the number of pollen parents in a mix to be identified and distinguished by markers? If accepting high analyzes costs per plant and large development costs for new loci an unlimited number of possible fathers can be distinctly recognized. But to start somewhere, it is better to look on what has been done by others before to find out what is realistic.

Wheeler et al. (2006) analyzed 16 parents and noted that although theoretically 0.9999 of the progeny could be uniquely assigned to a parent, only 95% was assigned. Morigushi et al (2005) have analyzed *Cryptomeria* seed orchards with wind pollinated maternal progenies with up to 69 clones by 6 SSR loci and with little problems. However, a large percentage of mislabeled clones were found. 100 loci were available and the most discriminating were chosen. Some studies use rather few clones or parents (Nilsen and Kjaer, 2006).

For Scots pine studies on the efficiency of markers for parental identifications has been done (Wang et al. 2009; Torimaru et al. 2009). Nine SRR loci have been developed and applied for Scots pine. The father could be identified among 28 seed orchard parents in seed harvested from one ramet in around 98% of the cases. The effort needed for routine analyses in a developed functioning system was claimed to be 2000 genotype analyses per Man-Year.

Based on these references it seems rather safe to claim that fathers can be identified in pollen mixes with 25 clones. Higher resolution means that more system has to be used and the analyses will be more expensive. More time has to be spent on the development of systems. I do not see 50 as unrealistic, but I claim it is neither optimal, nor needed for intentional mixes. Better systems will develop over time, but I do not recommend trusting in this unknown development when deciding the number of pollen in mixes today.

## **Using polymix breeding for estimating paternal breeding values**

It is possible to estimate breeding values from pollen parents based on polymix breeding. If a random sample of phenotypes get their paternal identity determined by markers, their breeding value can be estimated based on the performance of trees with a pollen parent. Or a fraction (usually larger than candidates required for forward selection) of the best performing phenotypes can get their father identified, and when breeding values of fathers for “ability to give good progeny” can be estimated.

The number of offspring per male parent will be somewhat more variable than the number of offspring per female, but this is taken care of by the BLUP analysis. Algorithms can be formulated to estimate breeding values on truncated samples of trees. An example of such truncated population is that the 10% of the phenotypes in each selected PMX family are genotyped, and the pollen parent breeding values are based only on these values.

It is probably regarded expensive to determine the father on a sufficient number of trees which are not candidate for selection to determine breeding values, and thus probably not that good idea.

## **Polymix breeding using fathers with known high BV**

A polymix can be composed from genotypes, which already have been tested and are known to have a high breeding value. They could come from an early compartment of the breeding population or a previous generation. The aim is not to make gain by selecting among the fathers, but only among the mothers. The strategy still gives room for e.g. linear deployment of fathers in the mix or in the selections. The progeny will serve as a recruitment population where a gain is made by selection on the mothers and phenotypic selection within the maternal progenies, while the function of the marker analyses is mainly to keep the balance between contributions from different parents. This can be a good alternative in situations, where the breeding within a subpopulation has not been synchronized, but grafts from tested clones from which pollen can be harvested are available in the same time as grafts from untested candidates become available.

## **Phenotypic selection is in itself powerful**

Phenotypic selection without knowing or identifying the parents is surprisingly powerful (Lindgren & Wei 2007, and literature cited there), if the breeding value of selections in the offspring is increased by using knowledge about the breeding value of the parents, that increase is eaten up by a corresponding increase in relatedness among selections. It has been suggested as a method for pre-selection of candidates in a situation where the progeny origin from open pollination in a seed orchard and the fathers of the candidates are determined by markers (El-Kassaby & Lindgren, 2008). These studies give strong hints that phenotypic selection of candidates in selected polymix families with subsequent parental analyses to get reasonable paternal contributions will be effective. Some further sophistication could be done by adjusting the number of candidates selected to the average of the family (thus the breeding value of the mother).

The offspring of good pollen parents will be more likely to be represented among the good phenotypes pre-selected, thus there will be a selection among pollen parents. If few pollen parents are used, this selection potential cannot be used, the marker analyses just decrease the gain by forcing larger balance among pollen parents, but if an excess of fathers are used, less pre-selections has to be discarded.

## **Polymix breeding combines the advantages of progeny-testing and phenotypic selection**

The combination of the possibility to estimate breeding values of the seed parents and to select among their progeny combining the seed parent breeding value and the phenotype and the paternal information strengthens this variant of polymix breeding very much compared to the situation when the PMX was used only to estimate breeding value of mothers. In the dead end progeny test case half of the genotype of the seeds (the one from the pollen parent) is just annoying random noise. The polycross breeding can be replicated each cycle pressing cycle time to 20 years.

## **Polymix in compartments**

Polymix could be based on compartments of a breeding population. For a Swedish breeding population with 50 founders where pre-selections of candidates has been done in F1 progeny and are available for crossing it could be something like the following: The breeding population is shared in say five compartments. Progeny from about 20% of the founder population are used as pollen parents in a polymix with 25 pollen. From another about 20% 50 pre-selections are used as seed parents for that pollen mix. Each compartment contributes a pollenmix and seed parents, 75 for each compartment. That is 325 for the whole breeding population shared in five compartments. The numbers or compartment size may not be the same and adjusted to availability and to get convenient units. One of the reasons for sharing in compartments is to get manageable pieces. Another is to make it easier to avoid strong unbalance at the end. Another is to get many candidates as fathers, but still not make the polymix mixture larger than 25. The fact that different females receive different pollen mixes does not greatly reduce the gain possible as long as there are sufficient many pollen in the mix, and that the pollen in different mixes do not origin from different populations (Lindgren, 2009), which may be a difficulty with the arrangement.

Pollen batches and seed parents should probably be matched to avoid inbreeding. A major selection is meant to be among maternal half sib families. Thus, it could be an advantage to keep these families together when organizing seed parents and pollen parents in batches, and they could constitute blocks in the field.

## Seed orchards

Seed orchard owners probably prefer a tested clone to a genotype with higher predicted breeding value, but represented only by a single tree on a single site around 13 years old. The decision if the alternatives are offered cannot be foreseen, but it feels safer to have both options available. It is also not possible to get many scions suitable for grafting from a 13 year old tree, while it may be easier to get scions from a graft of a progeny-tested tree. Polymix breeding offers both alternatives. Single or Double Pair Mating also results in that parent get a breeding value estimate, but polymix breeding results in more reliable results (Lindgren, 2009). Polymix breeding can be the best available alternative if considerable weight is put to the ability to produce tested clones for seed orchard with a higher gain in long term breeding than a progeny test based breeding strategy.

## Swedish Scots pine specific considerations

The current Swedish breeding strategy is based on mainly balanced selection, “within family selection”. Full balance does not go well together with polymixbreeding since selected fathers will be rather unequally distributed among the best phenotypes and some pollen parents may be impossible to get represented. Polymix-breeding is relatively more favourable if a considerable unbalance is accepted.

Almqvist (2009) has time scheduled Swedish activities (south Sweden). This polymix breeding alternative is timed and plant resources are set according to Almqvist alternative 5, which is probably the most correct comparison. The cycle start with top-grafting

Table 1.  
Time components in the breeding cycle.

Step	Time (years)
Top-grafting of selections	3
Crossings to get PMX families	3
Plant production	1
Field test	13
Pre-selection; marker analyse; selection of breeding population	
Cycle is complete, go to topgrafting for next cycle	
Cycling time	20

It will be hard or impossible to get time for measurement; evaluation; pre-selection; marker analyses; evaluation; final selection and collection of scions without addition of an extra year.

A Swedish polycross breeding alternative for further considerations could look like follows. We start the cycle in an  $F_1$  field trial originating from pair crosses of 50 parents (routine in the Swedish breeding program of Scots pine) by selecting and top-grafting 375 trees at age 13 years. Three years later the crosses can commence. Pollen parents are arranged in five different pollen batches (polymixes) comprising 25 pollen each. Five compartment of females, each 50 females, 250 in total, are pollinated with a polymix. These 250 half-sib families are grown in field trials (25 000 seedlings). At age 13 a number of trees (800) are pre-selected and genotyped by markers. The criterion for the pre-selection is a combined index of the half-sib performance and the within family performance

with some constraining against too much relatedness. The fathers of the pre-selections are determined by markers. Then 375 selections to the breeding population are top-grafted. The breeding cycle is closed, the cycle time is 20 years. The cycle is repeated. This is an idea, but I do not think it is optimal and I recommend further thinking (including studying what is more in this workreport).

## **Polymix breeding has to be tested experimentally**

We may simulate in computers, but I guess reality here is more complicated than can be put into a simulator, and we can be confident enough that it works and is good to make at least one small scale practical application of polymix breeding. The long-term development is in favour of molecular (marker) uses and time is ripening to start to implement applications into the real breeding. To state it a little stronger: it seems almost unavoidable that DNA markers will be applied in breeding during the next decade for at least some applications in more advanced situations, to where Sweden belongs. And for curiosity reasons, since the pressure to try something new in molecular genetics is high, it becomes increasingly difficult not to have at least some actual practical experience to base discussions on. Polycross breeding requires additional qualified development, but that requires “sharp” materials to be meaningful, and it seems almost certain the development can be done.

The application of markers for parentage and ancestry identification is much more close to application than is the identified super genes, but once marker analyses is made it is relatively more easier to extend them to supergenes if more realistic applications would be suggested. Thus, I recommend that a project on marker identification in breeding is taken up for a share of one breeding population before 2012 (not to jump too fast into something breeding is not mature for I limit it to one case, as I may be overoptimistic). I also suggest a project is started in parallel to make many controlled crosses (“more parents than grandparents”) for comparison since I believe the later strategy may be better. So a comparative test is suggested in a compartment in the breeding population.

It is likely that pine breeding can be 50% more effective if the breeding population is made considerable larger than used for long term transmittance of genes (Lindgren et al. 2008), or to express it in another way it is favourable to use many more parents than grandparents. Polymix breeding would easily be adapted to fulfil that demand!

Polymix breeding is complicated and competence demanding, and should not be tried in a low-input situation with little access to the right competence or some marginal situation yet, and in a program which is not certain to survive over decennia, at least not as a starter. But once the competence and technique is developed and polymix breeding has proven its value, it may be considered over the whole range of breeding operations including more species.

## Polymix breeding for species where clone testing is possible

This discussion has mostly focused on pine, but the system may be considered for other species and situations also. Even if clone testing is superior to progeny-testing there it is possible, recombination is still needed and repeating the testing with progeny gives extra gain. It is also relatively easy to increase the breeding population size and apply the grandparental strategy (see above) Therefore replacing double pair mating with polycross followed by selection forwards in the polycross progenies may have advantages even in programs where clone testing is a part of the strategy. If polymix breeding becomes established in pine, I recommend test the method in spruce also.

**Remarks:** There is a patent associated with Polymix breeding (United States Patent 7378570) and a patent application useful for a detailed analyses and literature study (WO/2002/005628 ). The terminology chosen here is Polymix breeding with acronym PMX/WFA. The acronym was coined by the inventor. Polymix breeding without adding “with paternity analyses” may be misunderstood, but is reasonable short and give approximately the correct associations so it is used within this study.

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