

# Integrating Pollen-Based Assisted Migration and Rolling-Front Landscape Breeding for Forest Adaptation under Climate Change

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Faculty of Forestry  
and Wood Sciences



Workshop "Seed Orchard Design", Waldsieversdorf, Feb 2023



- <https://home.cznu.cz/en/lstiburek/home>
- <https://github.com/mlstiburek>
- [lstiburek@fld.cznu.cz](mailto:lstiburek@fld.cznu.cz)
- <https://www.evoltree.eu>



# Forest trees

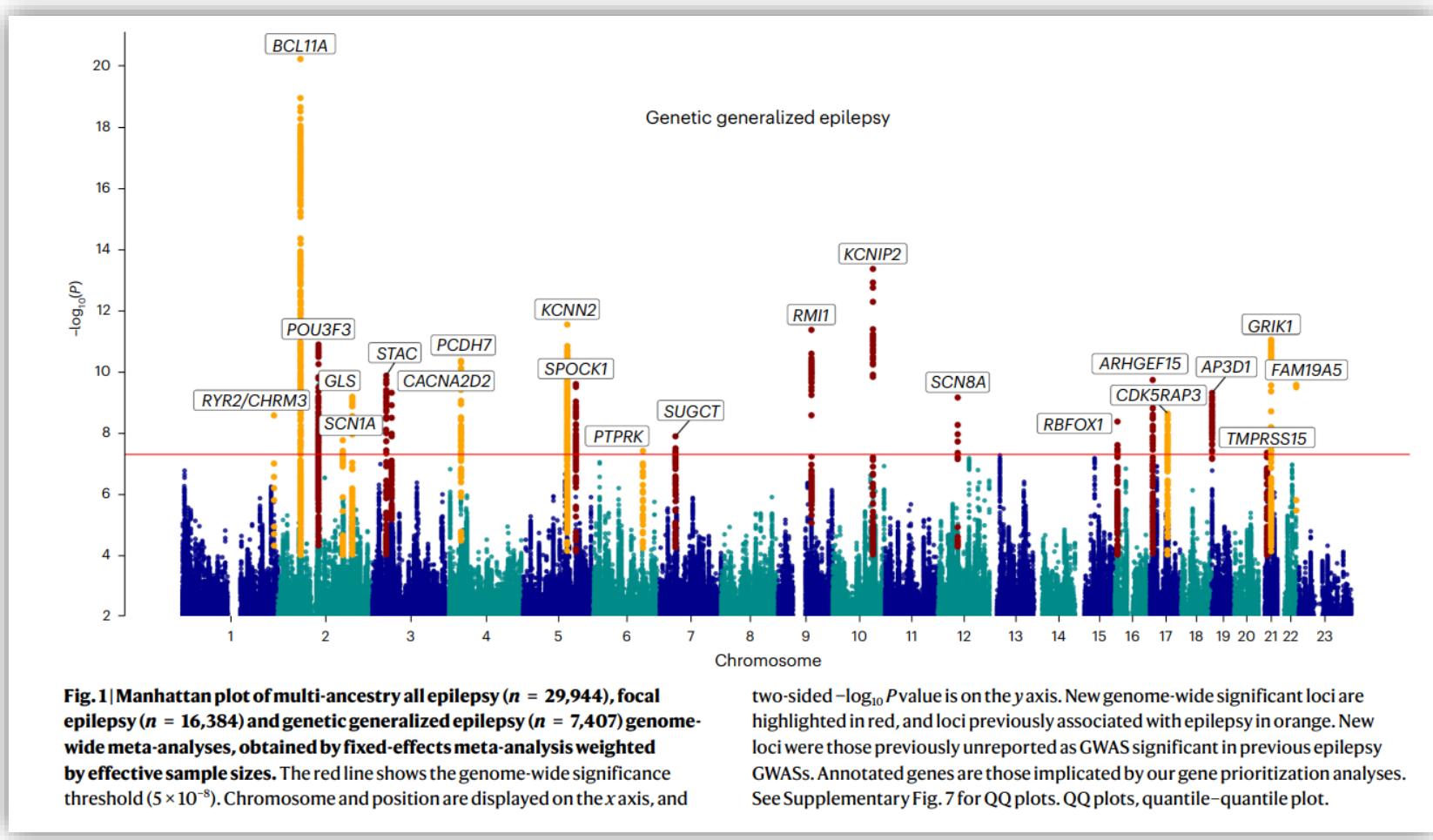
- Long generation intervals
- Large physical size and space requirements
- Heterogeneous environments
- Multifaceted forest utilization
- Undomesticated species

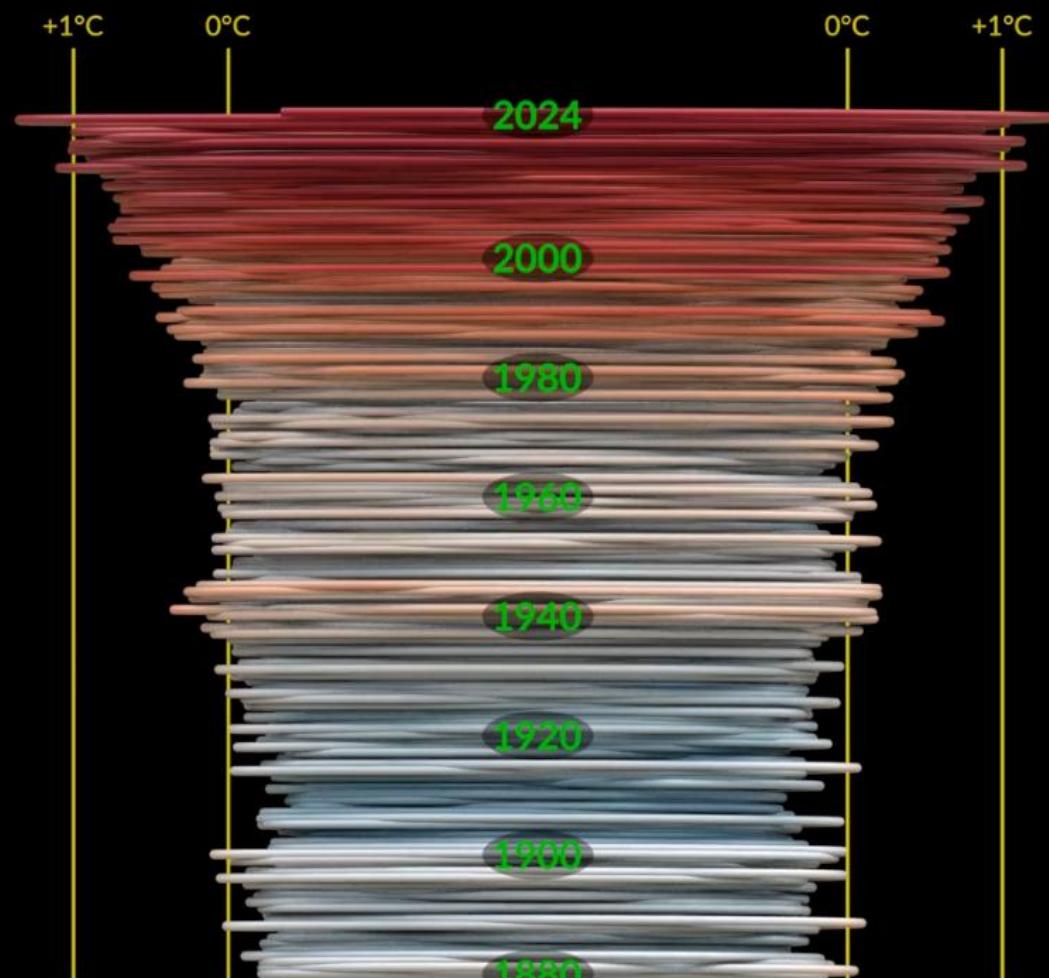
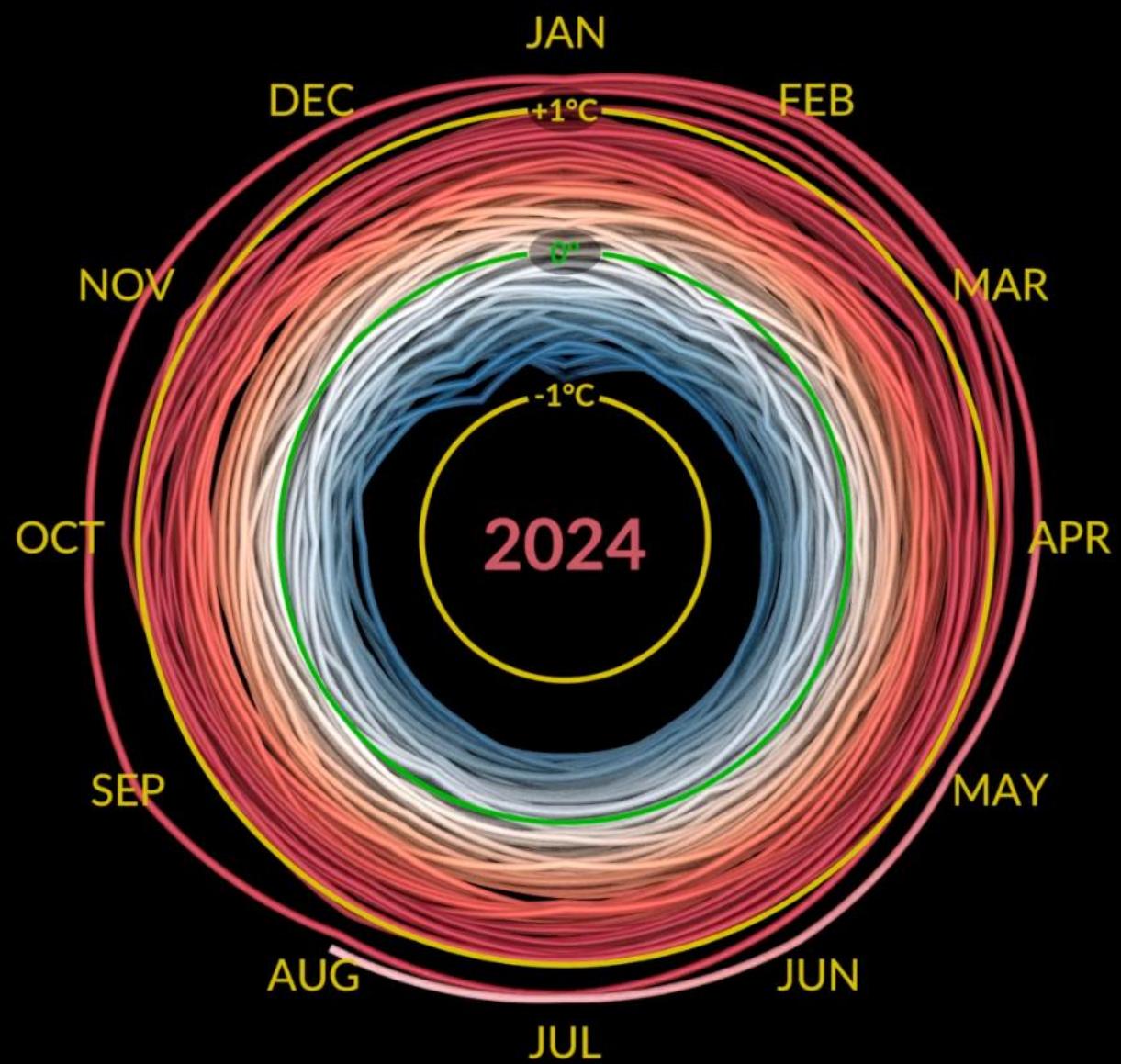


A photograph of a forest scene. In the foreground, there's a fallen tree trunk and some tall, dry grass. Behind it, there's a dense stand of young evergreen trees, likely pines or firs. In the background, a much denser forest of similar trees stretches across a hillside under a clear blue sky.

# Forest trees

- Mostly outbred species
- Genome size
- Complex traits expressed at late life stages
- Fast LD decay
- Low heritability
- GxE interaction
- Maintenance of high genetic diversity





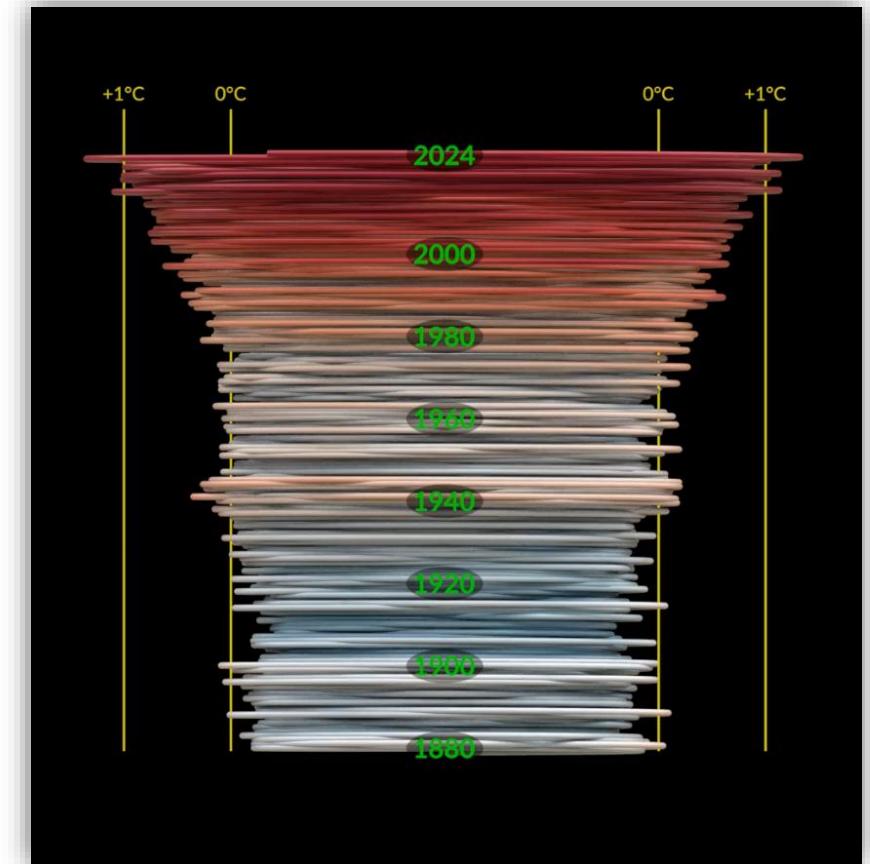
$$p = g + e$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

$$\sigma_g^2 = \sigma_a^2 + \sigma_d^2 + \sigma_{aa}^2 + \sigma_{ad}^2 + \sigma_{dd}^2$$

$$\sigma_e^2 = \sigma_{ec}^2 + \sigma_{eg}^2 + \sigma_{es}^2$$

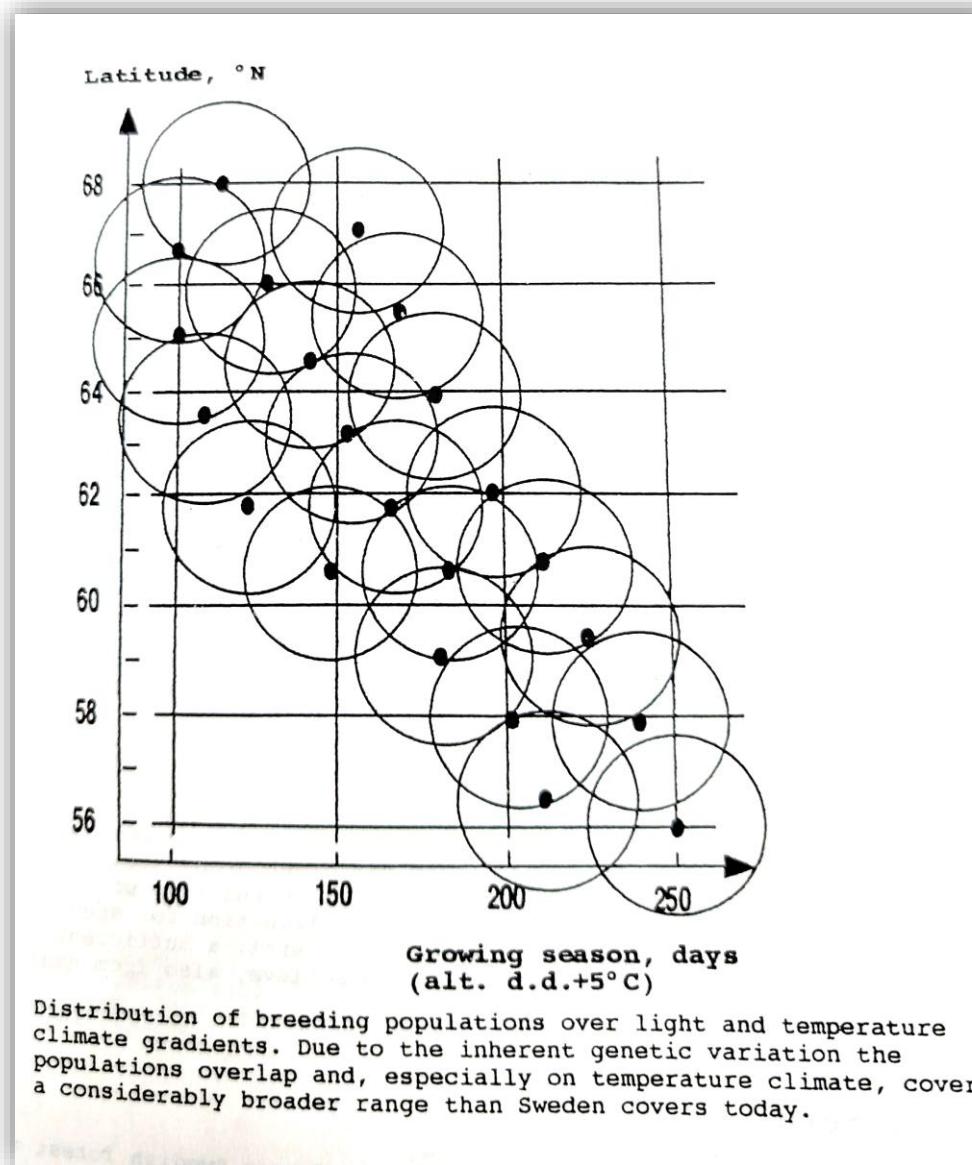
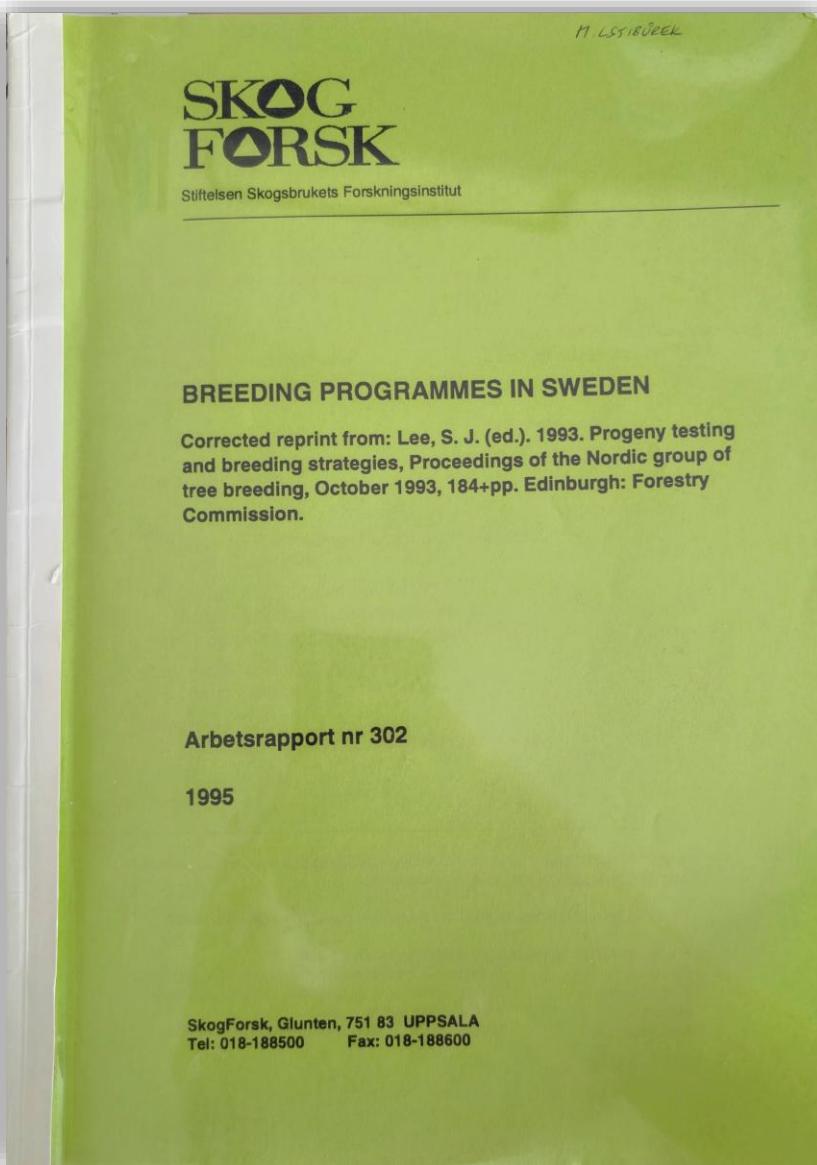
$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 + 2\text{cov}_{ge} + \sigma_{ge}^2$$



+ epigenetics

Wang Z. et al. (2012). A quantitative genetic and epigenetic model of complex traits. BMC Bioinformatics 13(1): 1-9.

# Breeding under climate change (> 30 years ago)



## Breeding without breeding

YOUSRY A. EL-KASSABY<sup>1\*</sup> AND MILAN LSTIBÚREK<sup>2</sup>

<sup>1</sup>Department of Forest Sciences, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

<sup>2</sup>Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, 165 21 Czech Republic

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### Summary

An innovative approach to tree breeding called ‘breeding without breeding’ (BWB) is presented. The method, as applied on the material in hand, allows the capture of 75–85% of the genetic response to selection attained through conventional programmes without the need to do any controlled pollination and simplified or possibly no experimental field testing: both considered to be the most resource-demanding activities in breeding programmes. BWB combines the use of genotypic or phenotypic pre-selection of superior individuals, informative DNA markers for fingerprinting and pedigree reconstruction of offspring to assemble naturally created full- and half-sib families resulting from mating among selected parents, and quantitative genetics analyses to identify elite genotypes for further genetic improvement or the establishment of production populations. BWB utility is demonstrated using a retrospective study of Douglas-fir (*Pseudotsuga menziesii*) progeny tests consisting of offspring produced from 150 controlled crosses among 60 parents and established over three sites. The empirical results are supported by theoretical expectations demonstrating anticipated minimum genetic response compared with conventional approaches. The method’s simplicity offers an exceptional opportunity for the development of comparable breeding efforts in developing countries, advanced and new breeding programmes, and economically important and ‘minor’ species.

### 1. Introduction

Forest gene resource management is a daunting task requiring balancing utilization and conservation goals (Yanchuk, 2001) for large, long-lived organisms covering vast ecological regions (Ying & Yanchuk, 2006). While the genetic improvement of economic traits is of primary importance, the maintenance of broad genetic diversity is essential to meeting changing breeding goals (Namkoong *et al.*, 1988) and guarding against unpredictable temporal and global environmental contingencies such as climate change (Eriksson *et al.*, 1993). Most tree improvement programmes follow recurrent selection schemes, consisting of multiple populations, including base populations where initial phenotypic selections are

made, breeding populations where crosses among the selected individuals are created and tested and deployment populations (e.g. seed orchards) harbouring elite genotypes for seed production (Namkoong *et al.*, 1988). Breeding arboreta are commonly established to safeguard the genetic legacy of original and subsequent selections and to facilitate breeding. Breeding populations are managed to maintain higher levels of genetic variability for sustained long-term genetic response while capturing genetic gain is the sole function of production populations (Namkoong *et al.*, 1988).

Predetermined mating designs are considered essential for creating structured, pedigreed families for genetic testing, facilitating accurate assessment of genetic parameters (breeding values, heritabilities, genetic gain, genetic variances and covariances and genotype–environment interactions), and the selection of elite genotypes for advanced breeding and seed orchards establishment (Lambeth *et al.*, 2001). Forest

\* Corresponding author. Yousry A. El-Kassaby, 3030-2424 Main Mall, Department of Forest Sciences, University of British Columbia, Vancouver, BC, Canada V6T 1Z4. Tel.: (604) 822 1821; Fax: (604) 822 9102. e-mail: y.el-kassaby@ubc.ca



PARENTS gen  $n + 1$

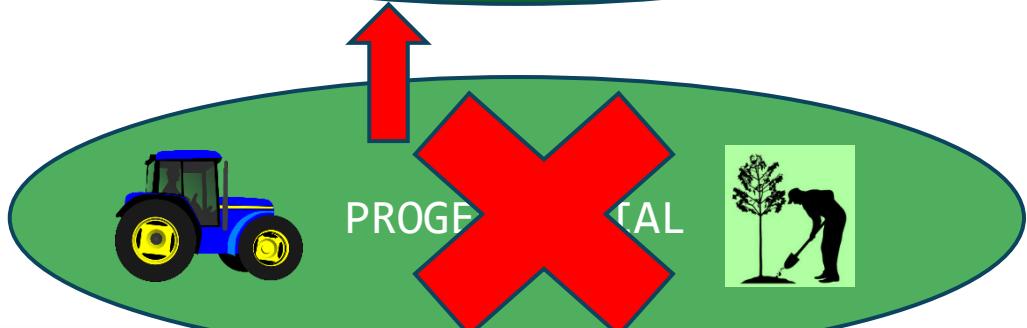


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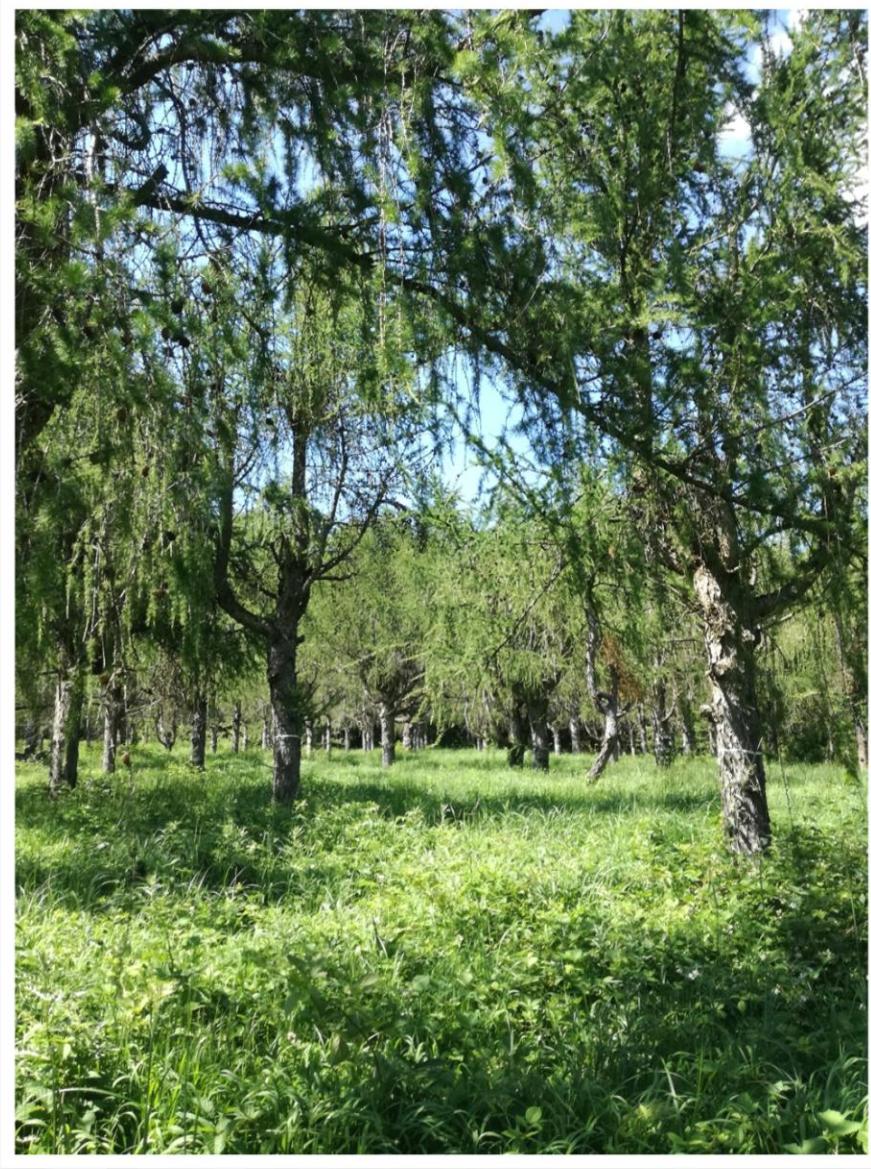
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# Project Lärche XXL

Austrian Research Promotion Agency (FFG)  
Forst Holz Papier (FHP) and LIECO nurseries  
and the Austrian Federal Forests (ÖBf)



1<sup>th</sup> gen seed orchard P3 (Hamet, Wienerwald, Austria)



Photo by courtesy of Petr Škorpík, BFW

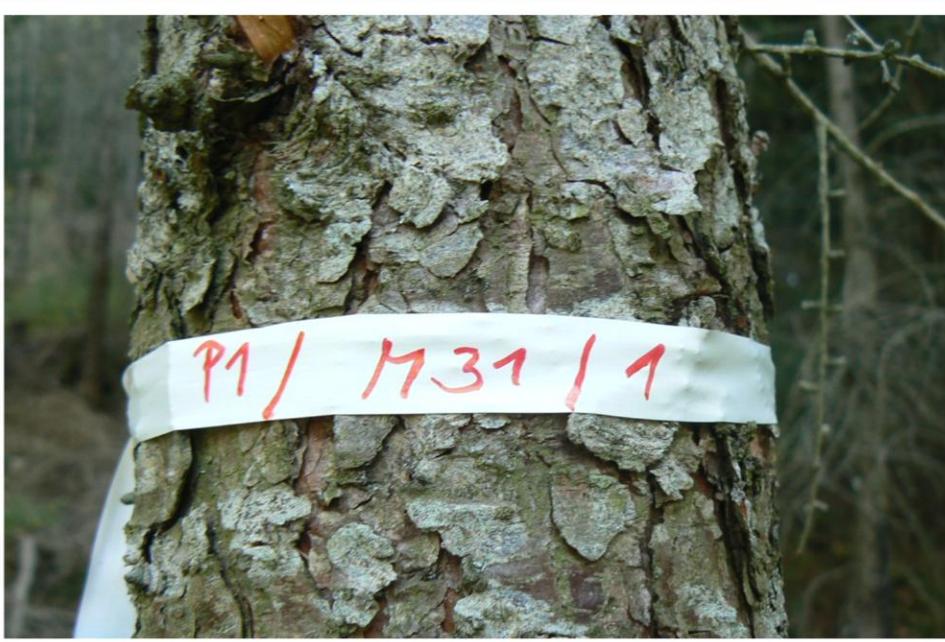
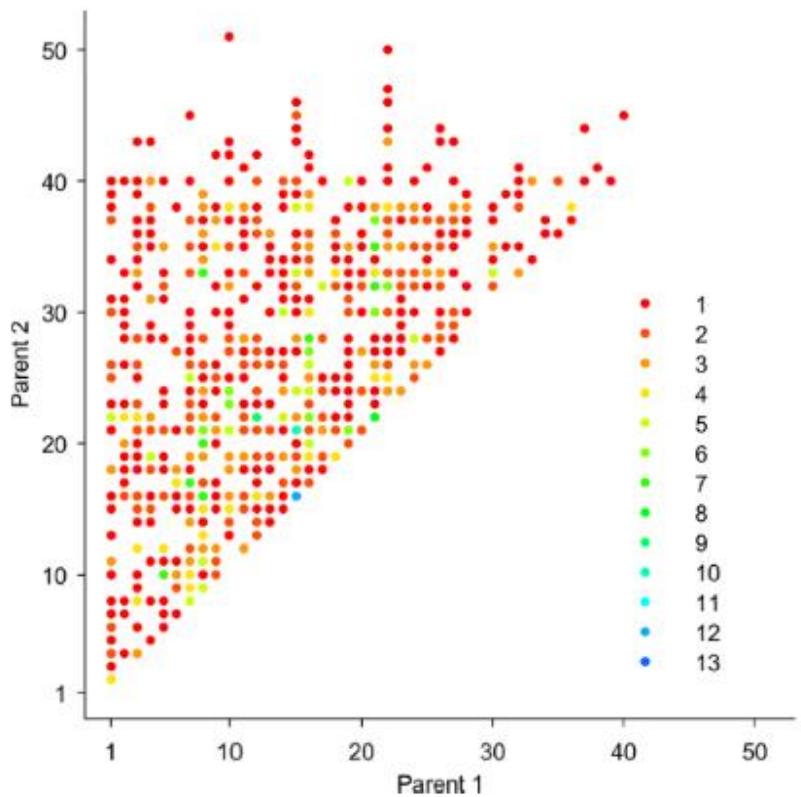


Photo by courtesy of Petr Škorpík, BFW



**FIGURE 2 |** Pedigree reconstruction results showing the formation of full-sib families and their parental combinations and respective family sizes (1-13, reciprocal crosses were grouped) ( $N = 1,024$  offspring).

- Novel *in situ* gene-resource management approach that conserves forest biodiversity and improves productivity and adaptation.
- No structured tree breeding/conservation methods.
- 4,267 25- to 35-year-old trees, 21 stands across 4 climatic regions in Austria.
- Pedigree reconstruction (17 SSRs), multi-trait, multi-site genetic analyses.
- Selection of broadly adapted and productive individuals.
- $h^2 0.23 \pm 0.07$  (height) and  $0.30 \pm 0.07$  (wood density) similar to conventional trials.



## In Situ Genetic Evaluation of European Larch Across Climatic Regions Using Marker-Based Pedigree Reconstruction

Milan Lstiburek<sup>1\*</sup>, Silvio Schueler<sup>2</sup>, Yousry A. El-Kassaby<sup>3</sup>, Gary R. Hodge<sup>4</sup>, Jan Stejskal<sup>1</sup>, Jiří Korecký<sup>1</sup>, Petr Skorpík<sup>5</sup>, Heino Konrad<sup>5</sup> and Thomas Geburek<sup>5</sup>

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#### Edited by:

Charles Chen,  
Oklahoma State University,  
United States

#### Reviewed by:

Jana Isabel Robab,  
University Institute of Psychological,  
Social and Life Sciences,  
Portugal

Richard Buggs,  
Queen Mary University of London,  
United Kingdom

\*Correspondence:  
Milan Lstiburek  
lstiburek@fsl.cz.czu.cz

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Photo by courtesy of Heino Konrad, BFW

2<sup>th</sup> gen seed orchard. Expected genetic response of 1.1 and 0.7 sd for fitness and productivity attributes, broadly adapted to a range of climatic conditions.

# Project Lärche XXL

2014: identification of forest stands, verification of origin

2015: measurements, DNA collection, lab analyses

2016-spring 2017: evaluation, selection, collection of scions

2019-2021: three new 2<sup>nd</sup> gen seed orchards (two in Aflenz, Frohnleiten, one in St. Martin im Innkreis)



## Rolling front landscape breeding

Milan Lstibùrek<sup>1\*</sup>, María Rosario García-Gil<sup>2</sup> and Arne Steffenrem<sup>3</sup>

### Key message

Forest tree breeding must undergo significant revisions to adapt to the evolving challenges posed by climate change. Addressing the shifts in environmental conditions requires a comprehensive multidisciplinary approach that includes theoretical work and practical application. Specifically, there is a need to focus on developing new breeding strategies that are theoretically sound and practically feasible, considering the economic constraints of actual tree breeding programs. We present a novel concept utilizing genetic evaluation of multiple traits in forest stands of successive ages across wide ecological ranges. Incorporating genomics allows for detailed genetic evaluation, making use of high-density SNP markers and sophisticated algorithms like GBLUP for genetic parameter estimates. High-throughput phenotyping is conducted using drone-borne lidar technology to capture tree height and survival data across various forest stands. Assisted migration is considered to strategically position genotypes across predicted environmental climatic gradients, thereby accommodating the dynamic nature of ecological shifts. Mathematical optimization acts as an essential component for logistics, guiding the spatial allocation and timely substitution of genotypes to ensure a continually adaptive breeding program. The concept replaces distinct breeding cycles with continuous evaluation and selection, enhancing the rate of genetic response over time.

**Keywords** Climate change, Adaptation, Gene diversity, Tree improvement, Genetic evaluation, *in situ* selection

### 1 Background

Genetic improvement of commercially important forest trees often involves long-term intensive recurrent selection programs requiring substantial resources and infrastructure. Breeding typically entails controlled pollinations creating structured pedigrees with offspring evaluated in replicated test sites within fixed ecological boundaries (zones). Production populations (typically seed orchards, SOs) are established at each selection

cycle as sources of genetically superior forest reproductive material for afforestation (White et al. 2007).

Following the development of DNA markers, forest geneticists have proposed various ways to incorporate kinship analyses into their programs. The main incentive has been to avoid laborious control crosses and shorten generational intervals, thus boosting genetic response to selection. In addition, progeny testing can be simplified or entirely redirected to operational forest stands planted across broader environmental gradients. These advances alleviate the shortcomings of traditional programs that are spatially static and slow to respond to market demands and climate change (CC, Hanewinkel et al. 2013). Lambeth et al. (2001) suggested a technique called polymix breeding with parental analysis, which was designed to be more cost-effective than full-sib breeding and would enable the identification of male parents to control relatedness or create a full-sib mating pedigree. El-Kassaby and Lstibùrek (2009) extended this concept for open-pollinated populations, emphasizing that genetic gain could be made without

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\*Correspondence:  
Milan Lstibùrek  
lstiburek@fau.cz

<sup>1</sup> Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Praha 6 165 00, Czech Republic

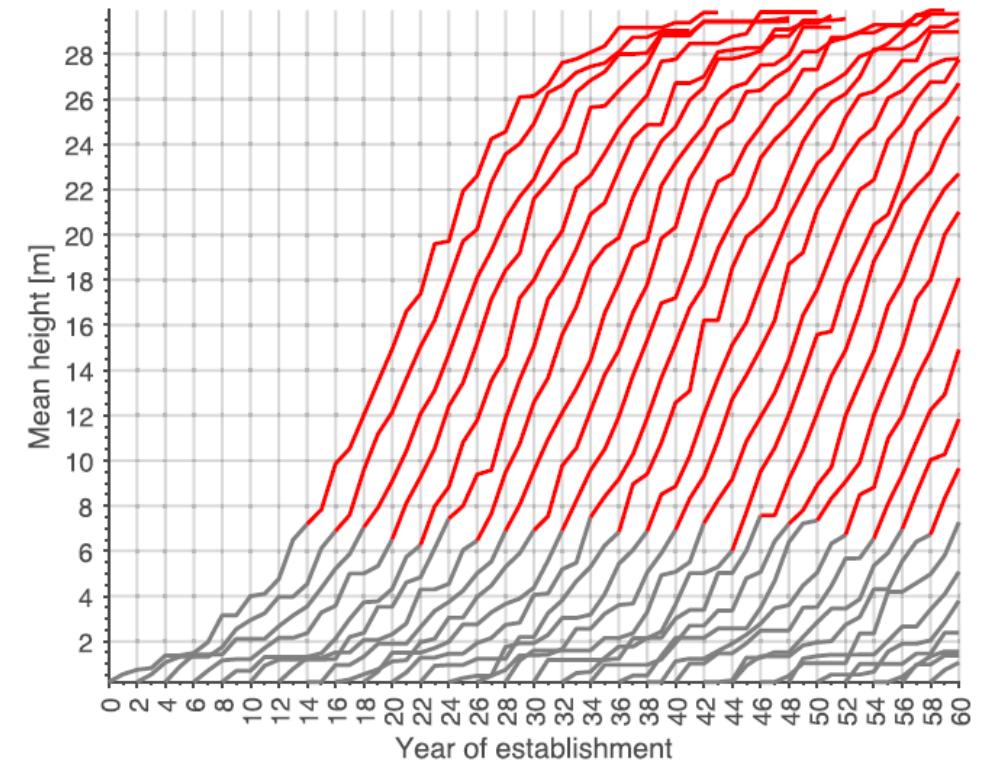
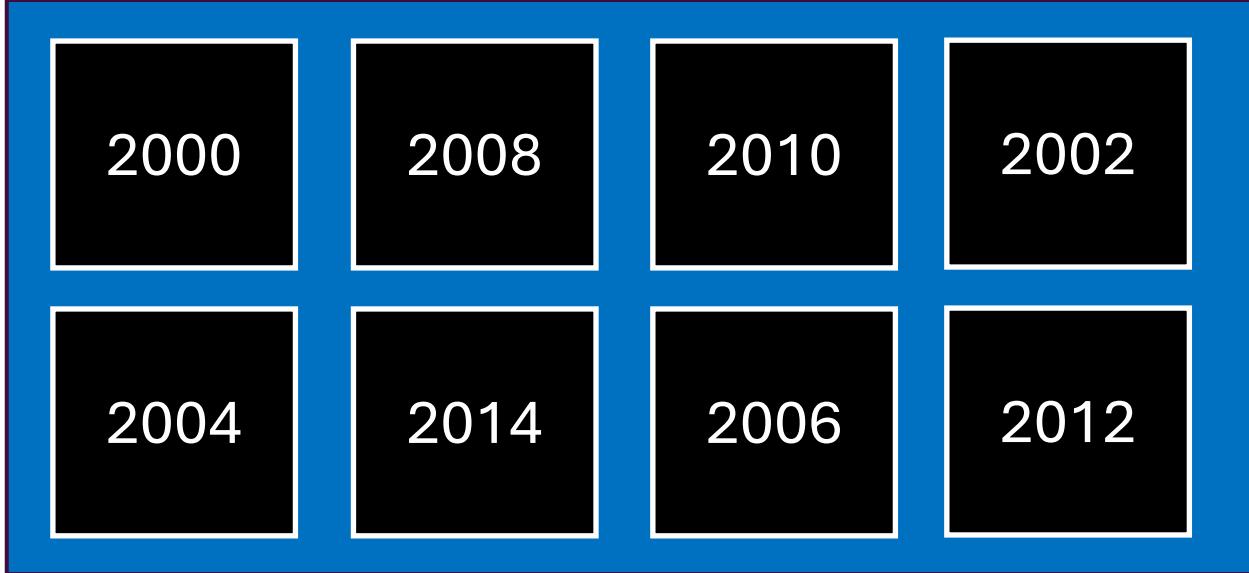
<sup>2</sup> Department of Forest Genetics and Plant Physiology, Faculty of Forest Sciences, Swedish University of Agricultural Sciences, Umeå 901 83, Sweden

<sup>3</sup> Norwegian Institute of Bioeconomy Research (NIBIO), Skogegata 22, 7713 Steinbjørn, Norway

Borralho, N. M. G. and Dutkowski, G. W., M. J. Dieters, A. C. Matheson, D. G. Nikles, C. E. Harwood, S. M. Walker editors (1996). **A rolling-front strategy for breeding trees.** Tree improvement for sustainable tropical forestry. QFRI-IUFRO Conference, Caloundra, Queensland, Australia, 27 October-1 November 1996. Volume 2., (317–322), Queensland Forestry Research Institute.

Borralho, N. M., & Dutkowski, G. W. (1998). **Comparison of rolling front and discrete generation breeding strategies for trees.** Canadian Journal of Forest Research, 28(7), 987-993.

Seed orchard  
(breeding arboretum)



Seed orchard

2000

2008

2010

2002

2004

2014

2006

2012

+12 years

Seed orchard

2000

2008

2010

2002

2004

2014

2006

2012

+14 years

Seed orchard

2000

2008

2010

2002

2004

2014

2006

2012

+16 years

Seed orchard

2000

2008

2010

2002

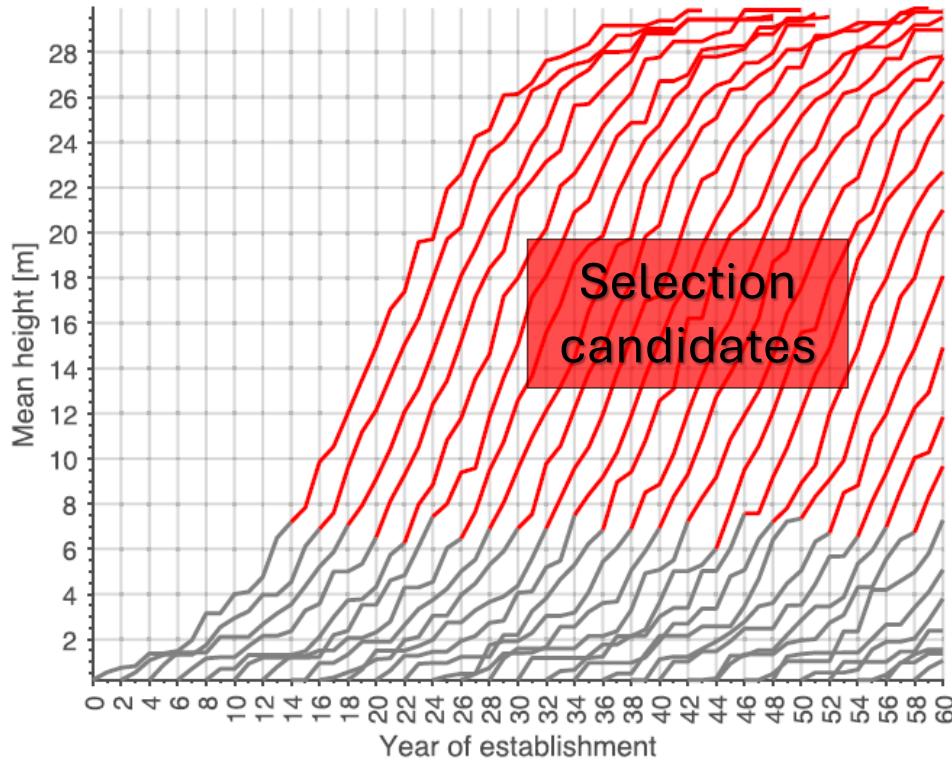
2004

2014

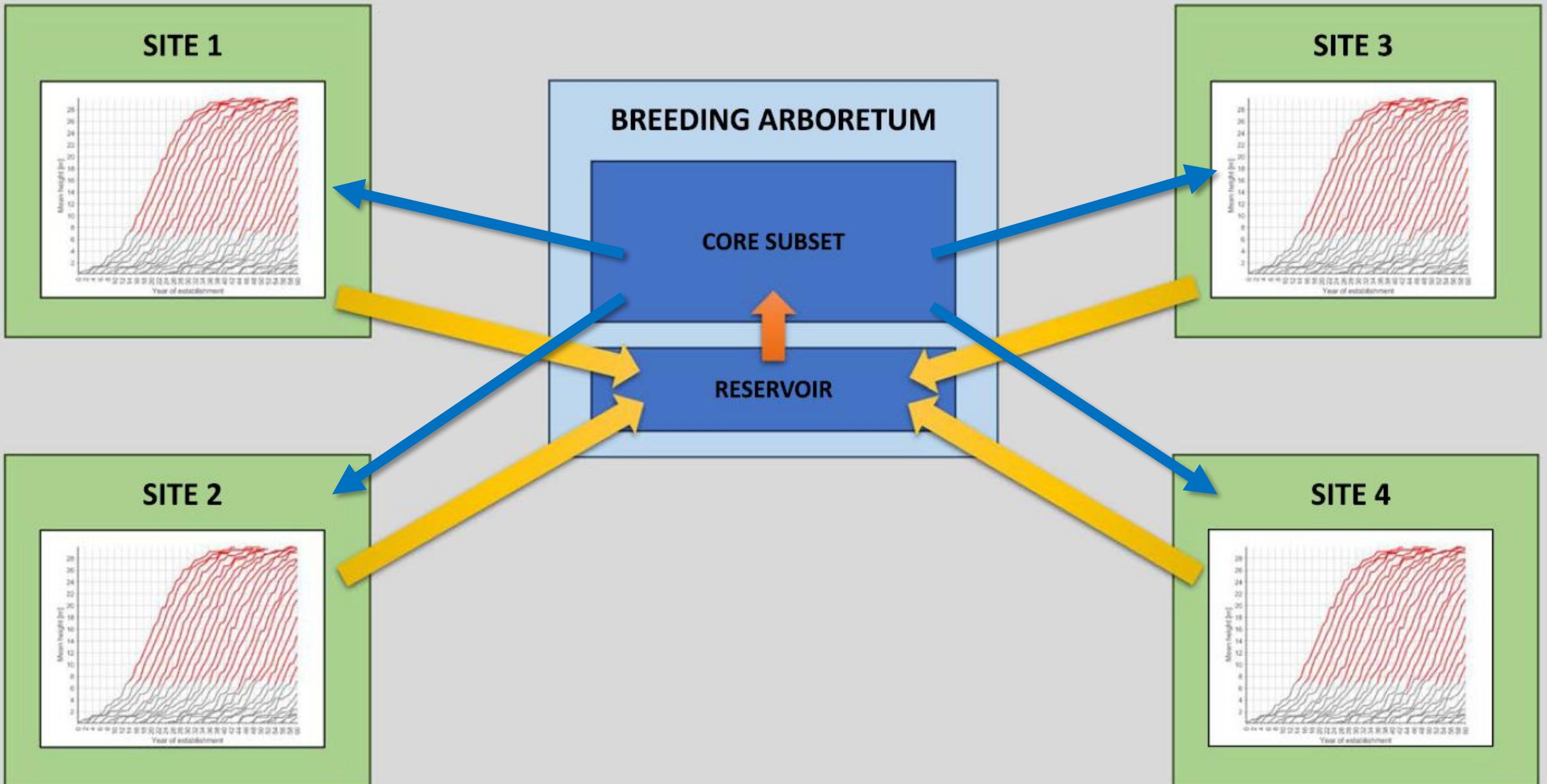
2006

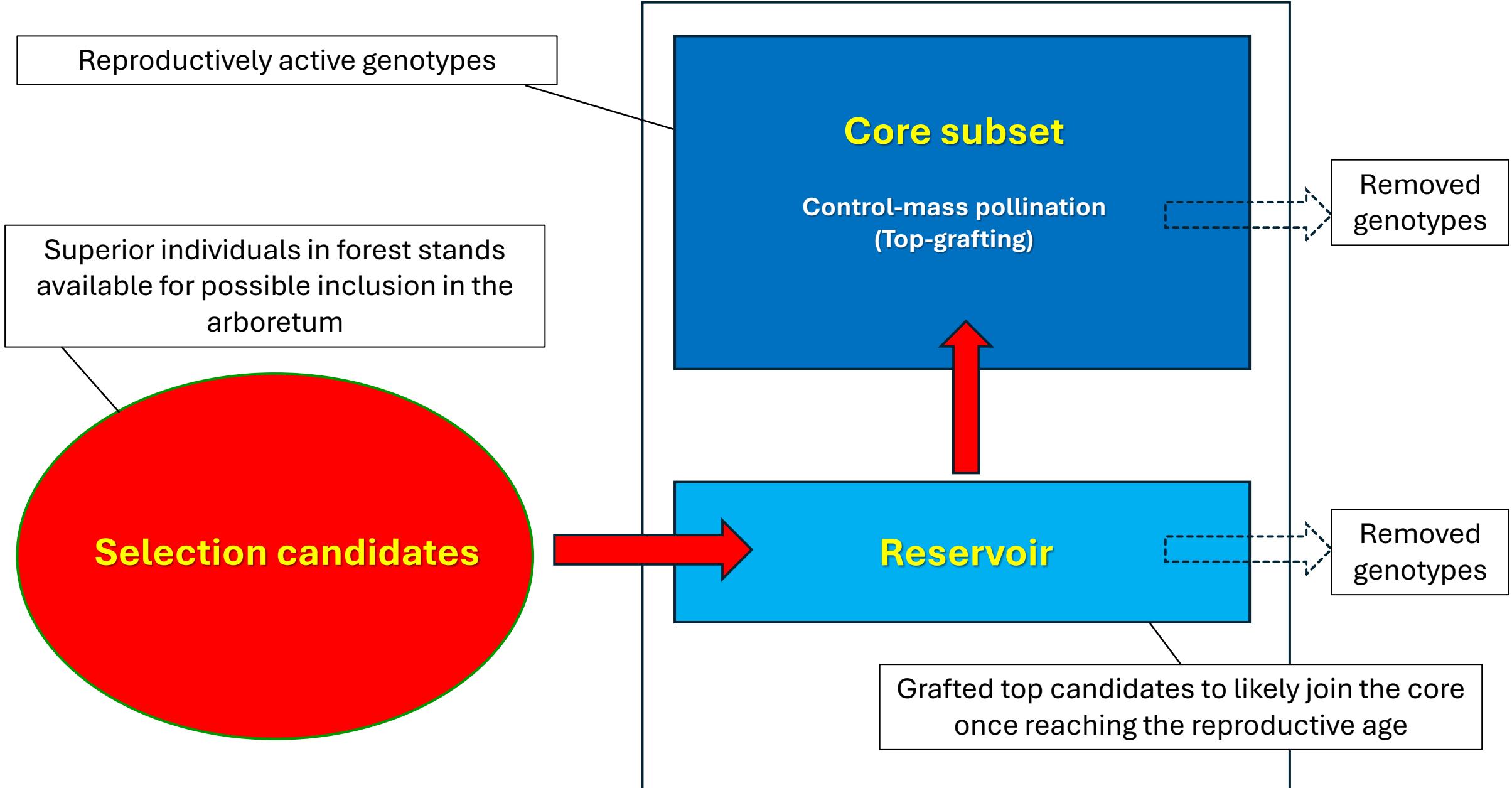
2012

+18 years



- each stand exposed to a **unique set of climatic conditions**  
→ specific **life stages** exposed to unique conditions!
- all stands originate from the **same parental source**
- **multi-site, multi-age** genetic evaluation







Ekebo - Skogforsk - Forestry Research Institute of Sweden

## Estimating Realized Heritability in Panmictic Populations

Milan Lstibůrek,<sup>\*†</sup> Václav Bittner,<sup>\*</sup> Gary R. Hodge,<sup>\*</sup> Jan Picek,<sup>\*</sup> and Trudy F. C. Mackay<sup>§</sup>

<sup>\*</sup>Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, 165 21 Praha 6, Czech Republic, <sup>†</sup>Faculty of Science, Humanities and Education, Technical University of Liberec, 461 17 Liberec 1, Czech Republic, <sup>‡</sup>Camcore, Department of Forestry and Environmental Resources and <sup>§</sup>Department of Biological Sciences and Program in Genetics, North Carolina State University, Raleigh, North Carolina 27695

ORCID IDs: 0000-0002-6304-6669 (M.L.); 0000-0003-3554-9602 (V.B.)

**ABSTRACT** Narrow sense heritability ( $h^2$ ) is a key concept in quantitative genetics, as it expresses the proportion of the observed phenotypic variation that is transmissible from parents to offspring.  $h^2$  determines the resemblance among relatives, and the rate of response to artificial and natural selection. Classical methods for estimating  $h^2$  use random samples of individuals with known relatedness, as well as response to artificial selection, when it is called realized heritability. Here, we present a method for estimating realized  $h^2$  based on a simple assessment of a random-mating population with no artificial manipulation of the population structure, and derive SE of the estimates. This method can be applied to arbitrary phenotypic segments of the population (for example, the top-ranking  $p$  parents and offspring), rather than random samples. It can thus be applied to nonpedigreed random mating populations, where relatedness is determined from molecular markers in the  $p$  selected parents and offspring, thus substantially saving on genotyping costs. Further, we assessed the method by stochastic simulations, and, as expected from the mathematical derivation, it provides unbiased estimates of  $h^2$ . We compared our approach to the regression and maximum-likelihood approaches utilizing Galton's dataset on human heights, and all three methods provided identical results.

**KEYWORDS** quantitative genetics; Hardy-Weinberg equilibrium; panmictic population

**N**arrow sense heritability ( $h^2$ ) is a key concept in quant- for common diseases in humans in the context of precision

- To accurately estimate narrow-sense heritability, it is sufficient to perform phenotyping and genotyping **only in the top phenotypic segment**.
- This approach **significantly reduces costs** by eliminating the need for a random population subset, as initially suggested by El-Kassaby and Lstibůrek (2009).

## Breeding without breeding: minimum fingerprinting effort with respect to the effective population size

Milan Lstibůrek · Kristýna Ivanková · Jan Kadlec · Jaroslav Kobliha · Jaroslav Klápník · Yousry A. El-Kassaby

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**Abstract** We present a probabilistic model to minimize the fingerprinting effort associated with the implementation of the “breeding without breeding” scheme under partial pedigree reconstruction. Our approach is directed at achieving a declared target population’s minimum effective population size ( $N_e$ ), following the pedigree reconstruction and genotypic selection and is based on the graph theory algorithm. The primary advantage of the proposed method is to reduce the cost associated with fingerprinting before the implementation of the pedigree reconstruction for seed parent-offspring derived from breeding arboreta and production or natural populations. Stochastic simulation was

conducted to test the method’s efficiency assuming a simple polygenic model and a single trait. Hypothetical population consisted of 30 parental trees that were paired at random (selfing excluded), resulting in 600 individuals (potential candidates for forwards selection). The male parentage was assumed initially unknown. The model was used to estimate the minimum genotyping sample size needed to reaching the prescribed  $N_e$ . Results were compared with the known pedigree data. The model was successful in revealing the true relationship pattern over the whole range of  $N_e$ . Two to three offspring entered genotyping to meet the  $N_e = 2$  while 41 to 43 were required to satisfy the  $N_e = 14$ . Importantly, genetic gain was affected at the lower limits of the genotyping effort. Doubling the number of parents resulted in considerable reduction of the genotyping effort at higher  $N_e$  values.

**Keywords** Breeding without breeding · Pedigree reconstruction · Selection

### Introduction

Unraveling the genealogical relationships among members of populations is of great importance to many aspects of biological sciences. The availability of numerous highly variable neutral DNA markers such as microsatellites coupled with the development of sophisticated mathematical methods for pedigree reconstruction (see Jones and Ardren 2003 for a review) has made this task manageable. Pedigree reconstruction has proven to be of substantial value to conservation biology, ecological genetics, and, more recently, has

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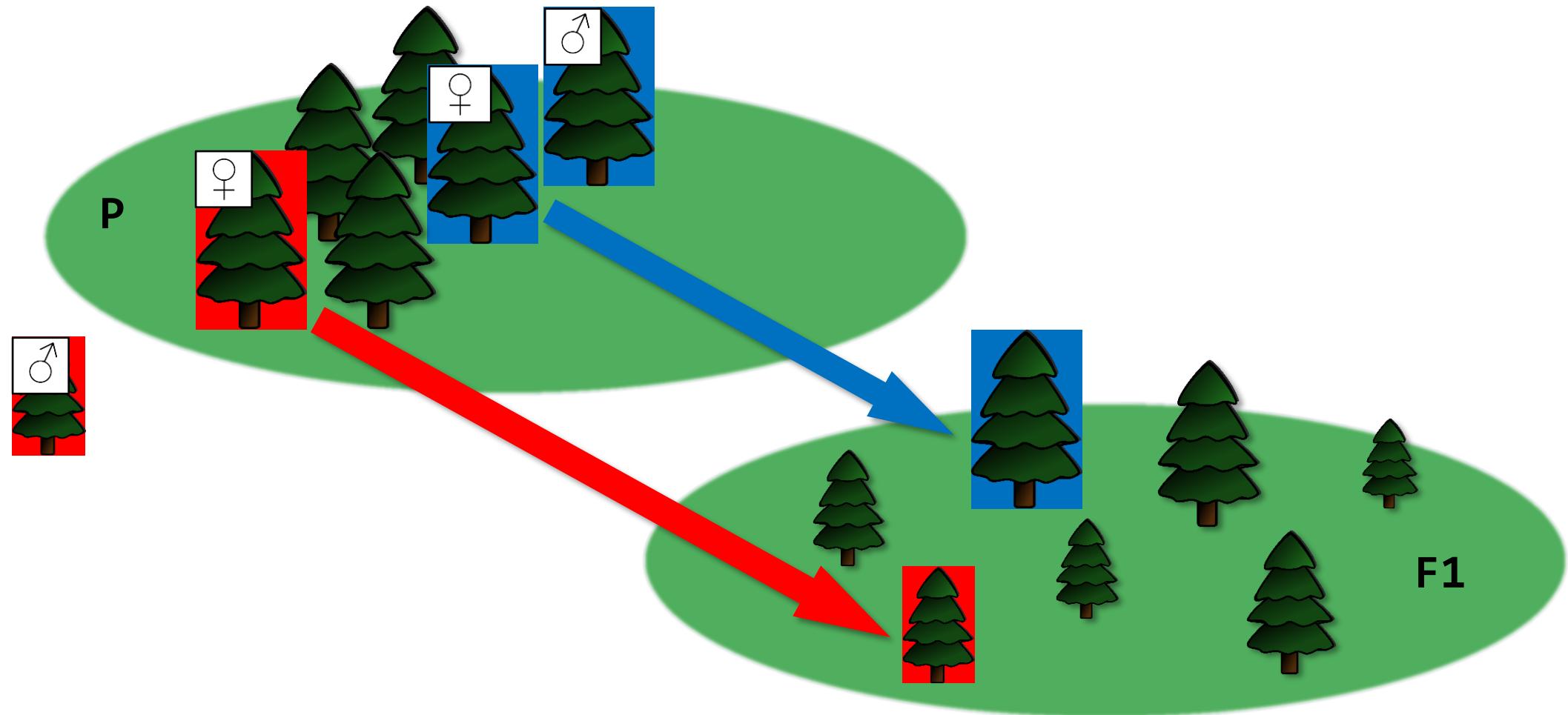
M. Lstibůrek (✉) · K. Ivanková · J. Kobliha · J. Klápník  
Department of Dendrology and Forest Tree Breeding,  
Faculty of Forestry and Wood Sciences, Czech University  
of Life Sciences Prague, Kamýcká 129,  
165 21 Praha 6, Czech Republic  
e-mail: lstiburek@fld.czu.cz

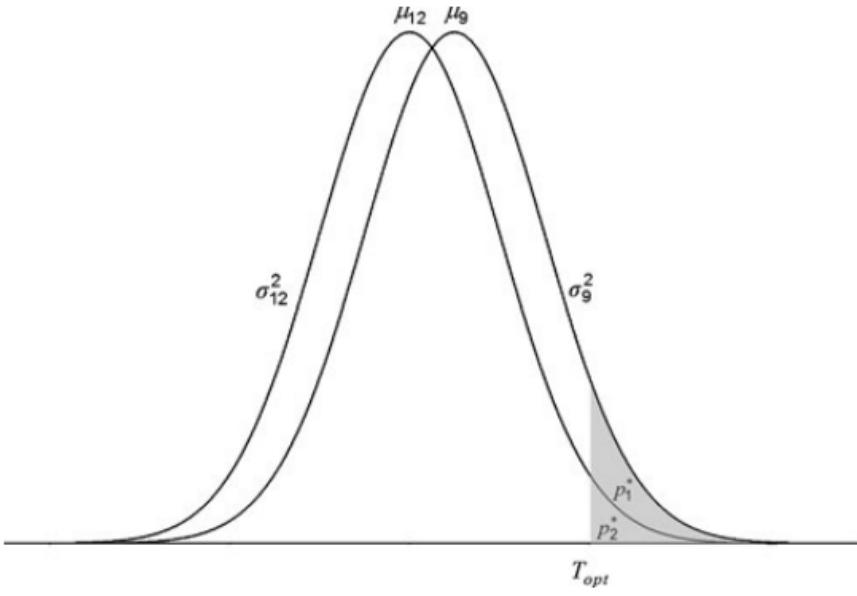
K. Ivanková  
Institute of Economic Studies, Faculty of Social Sciences,  
Charles University in Prague, Opletalova 26, 110 00 Praha 1,  
Czech Republic

J. Kadlec  
Faculty of Mathematics and Physics, Charles University  
in Prague, Ke Karlovu 3, 121 16 Praha 2, Czech Republic

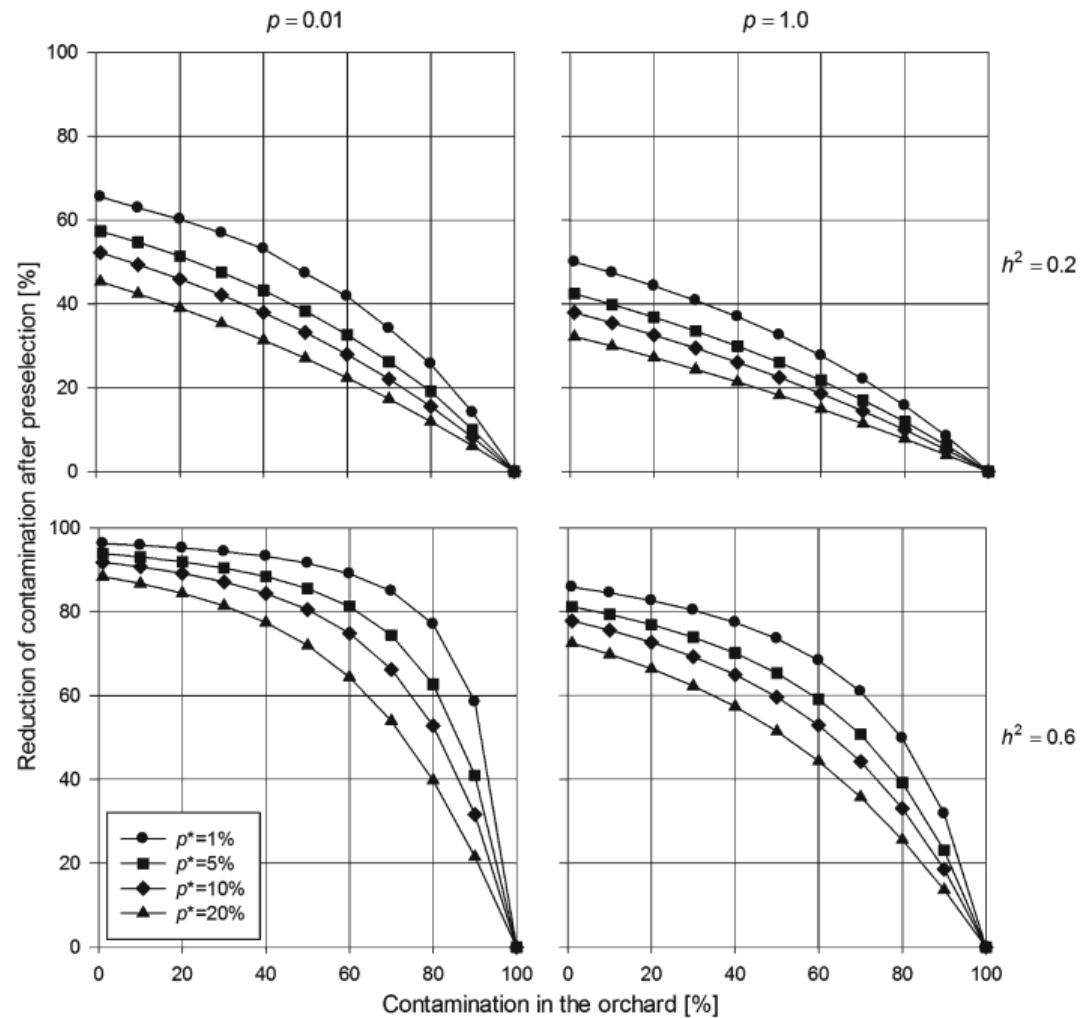
J. Klápník · Y. A. El-Kassaby  
Department of Forest Sciences, Faculty of Forestry,  
University of British Columbia, 2424 Main Mall,  
V6T 1Z4 Vancouver, BC, Canada

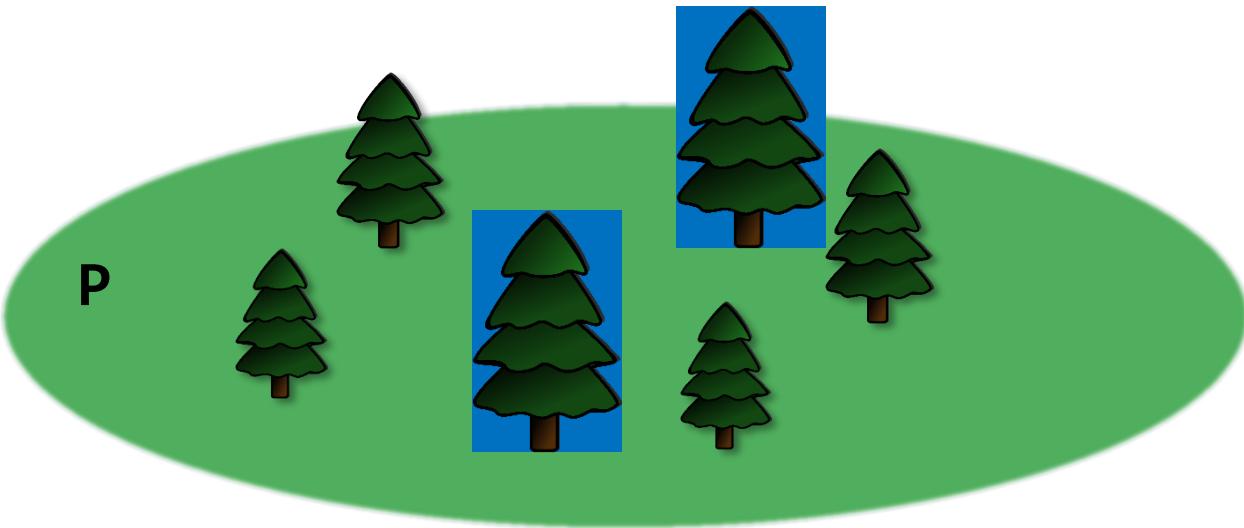
# Realized heritability in panmictic population



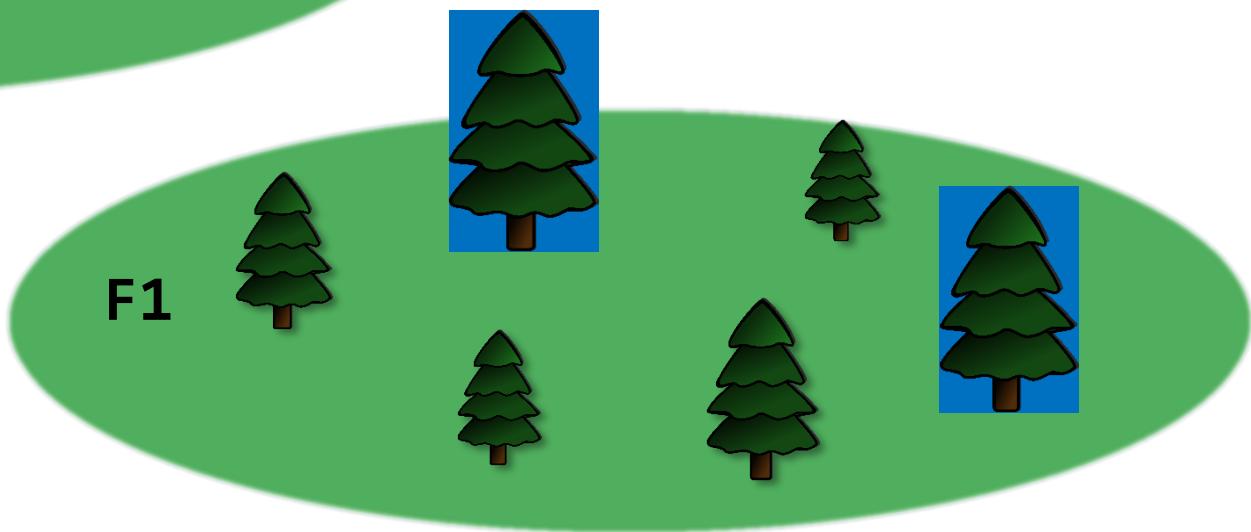


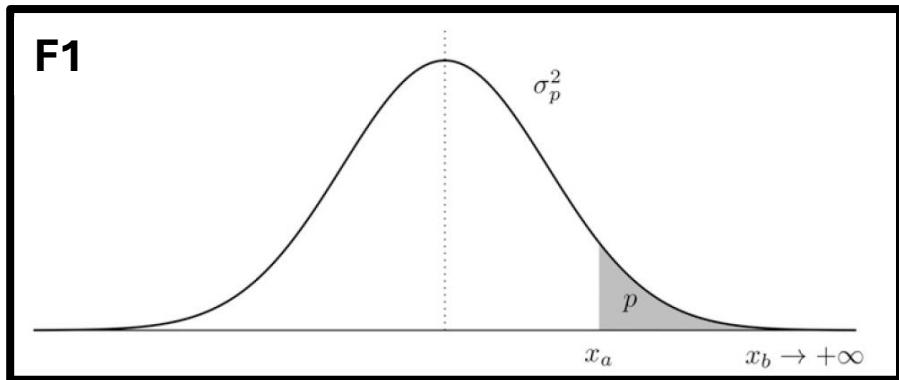
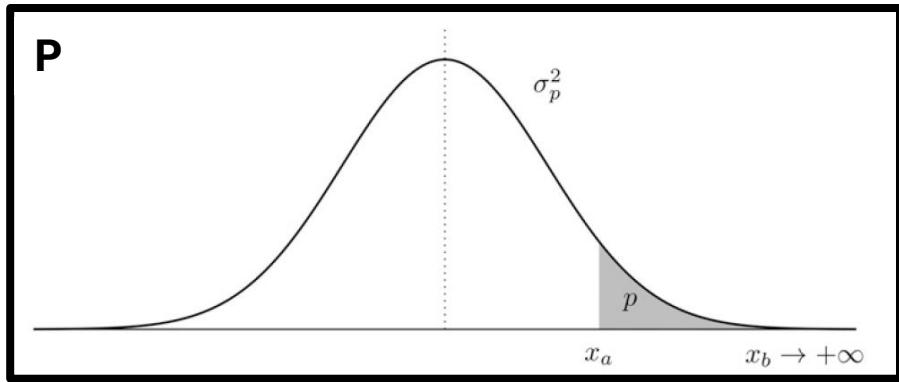
**Fig. 1** Conceptual representation of two offspring populations produced from within the orchard's pollen pool (right,  $\mu_9$  and  $\sigma_9^2$  corresponding to Eq. 9) and those sired by outside pollen (left,  $\mu_{12}$  and  $\sigma_{12}^2$  corresponding to Eq. 12). The area beyond the truncation point denotes the probabilities  $p_1^*$  (Eq. 9) and  $p_2^*$  (Eq. 12), representing within-orchard and outside pollination, respectively. The actual parameters (i.e., means and variances) depend on the input.  $T_{opt}$  is the truncation point produced by Solver optimization





?  $h^2$



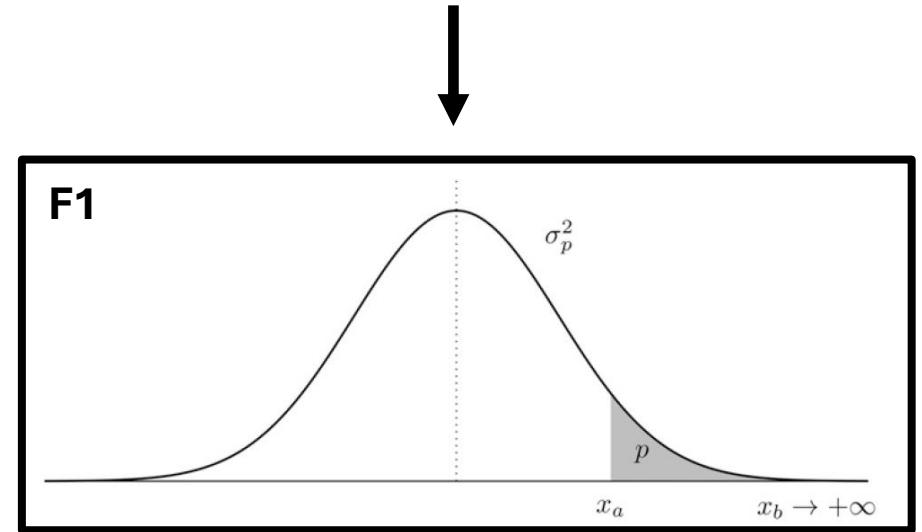
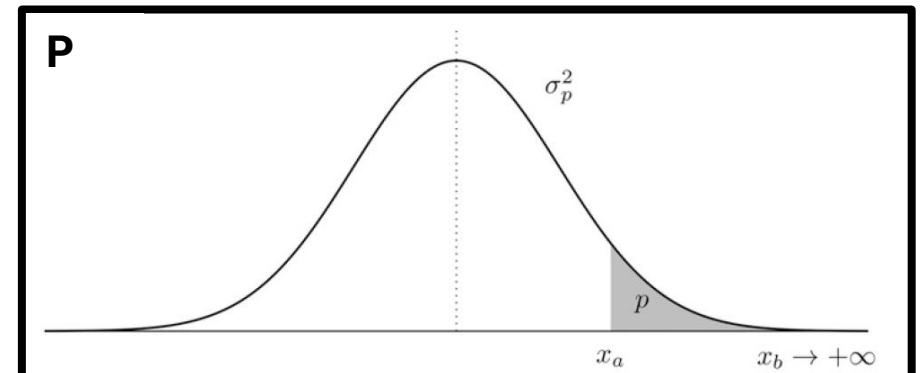


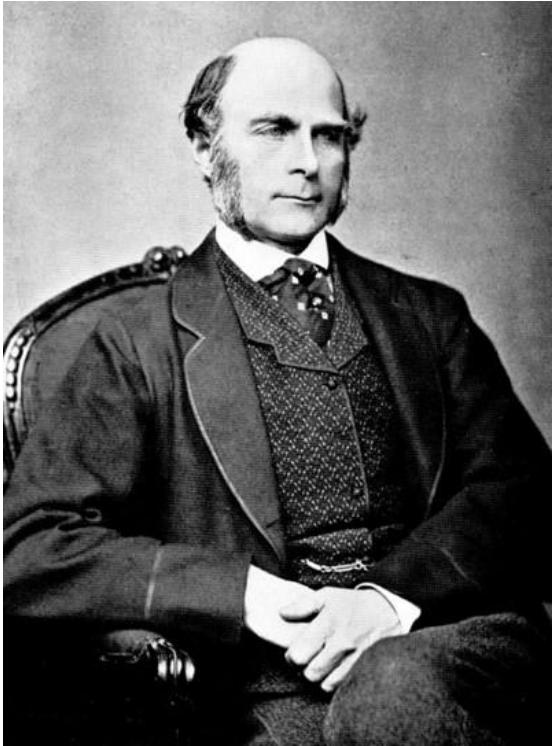
„Under the assumptions of Hardy-Weinberg equilibrium, the  $h^2$  of a quantitative trait in a given population is directly related to the likelihood of a phenotypic subset of parents passing their respective alleles onto the corresponding phenotypic subset of offspring.“

1. Estimate of phenotypic mean and variance in P and F1 populations.
2. Evaluation of whether respective parents originated in an equivalent truncated subset of the distribution. (optional pedigree reconstruction based on DNA markers).

$$h^2 = \frac{2\alpha Bi \pm [4\alpha^2 B^2 i^2 - 2(2B^2 i^2 + BQ'^2 k)(\alpha^2 - Q'^2)]^{\frac{1}{2}}}{(2B^2 i^2 + BQ'^2 k)}$$

$$SE\{h^2\} = \frac{(1 - 0.5h^4 kB)^{\frac{3}{2}}}{|-\phi(a)(-iB + 0.5h^2 \alpha kB)|} \frac{p}{P(Y)} \sqrt{r(1-r)} \frac{1}{\sqrt{n}}$$



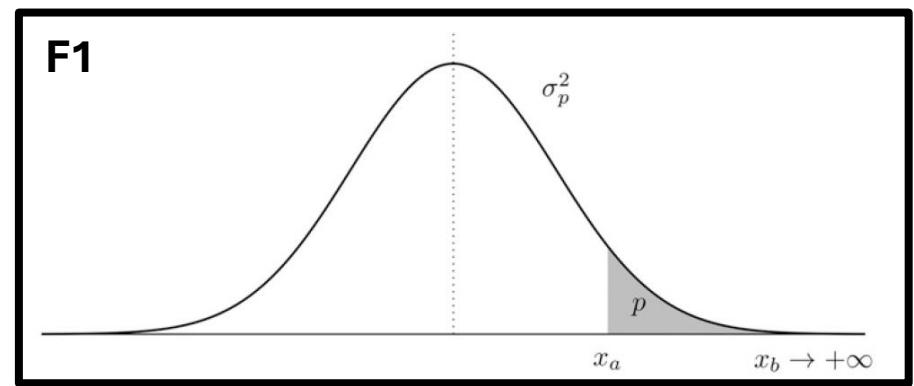
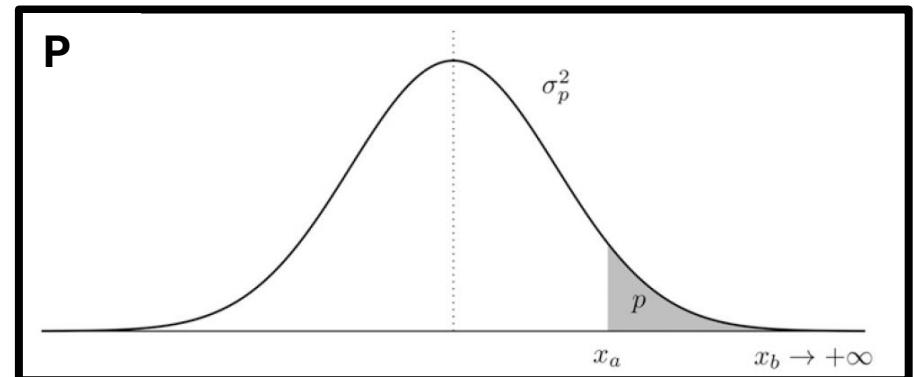


For demonstration, we used human height data collected by F. Galton. Datafile includes 205 families (934 offspring, range of family sizes: 1 to 15 individuals).



Galton F. (1886). Regression towards mediocrity in hereditary stature. *J. Anthropol. Inst. G. B. Irel.* 15: 246–263.

Estimates  $h^2 = 0.71 - 0.73$ , statistically equivalent with linear regression.



# Traditional tree breeding

Discrete generations,  
static environment

- Progeny test trials established once per cycle, within a narrow environmental window. Selection decisions made decades later, implicitly assuming GxE effects are stationary across time.

Climate blind spot

- Because climate is shifting, each discrete cohort only reflects a snapshot of past conditions. When breeding values are estimated within that static cohort, the estimates may be biased toward adaptation to an environment that may never recur.

# Rolling-front landscape breeding

## Frequent staggered recombination

- Instead of one large recombination event per 10-30 years, recombination is recurrent. Each cohort is exposed to unique environmental conditions (drought years, frost years, pest outbreaks, heat waves).

## Joint multi-cohort evaluation

- Simultaneous genetic evaluation across all sites and ages under a mixed-model framework.
- If one cohort encounters a severe drought or frost event, the response of its families contribute information to the breeding value estimation across the entire population. This allows capturing adaptation signals to extreme or novel climates.

## OPINION OPEN ACCESS

## A Pollen-Based Assisted Migration for Rapid Forest Adaptation

David Chludil<sup>1</sup> | Jaroslav Čepík<sup>2</sup> | Arne Steffenrem<sup>3</sup> | Jan Stejskal<sup>1</sup> | Christi Sagariya<sup>4</sup> | Torsten Pook<sup>5</sup> | Silvio Schueler<sup>6</sup> | Jiří Korecký<sup>1</sup> | Curt Almqvist<sup>7</sup> | Mats Berlin<sup>8</sup> | Arne Steffenrem<sup>1</sup> | Steven E. McKeand<sup>4</sup> | Jiří Korecký<sup>9</sup> | Jan Stejskal<sup>1</sup> | Jaroslav Čepík<sup>2</sup> | Fikret Isik<sup>4</sup> | Debojyoti Chakraborty<sup>3</sup> | Mats Berlin<sup>8</sup> | Milan Latibák<sup>1</sup>

<sup>1</sup>Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic | <sup>2</sup>Division of Forest and Forest Resources, Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway | <sup>3</sup>Animal Breeding and Genomics, Wageningen University & Research, Wageningen, The Netherlands | <sup>4</sup>Department of Forest Growth, Silviculture and Genetics, Austrian Research Center for Forests (BFW), Vienna, Austria | <sup>5</sup>Forestry Research Institute of Sweden (Skogforsk), Uppsala Science Park, Uppsala, Sweden

Correspondence: Milan Latibák (latibak@fid.czu.cz)

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## ABSTRACT

Climate change poses an unprecedented threat to forest ecosystems, necessitating innovative adaptation strategies. Traditional assisted migration approaches, while promising, face challenges related to environmental constraints, forestry practices, phytosanitary risks, economic barriers, and legal constraints. This has sparked debate within the scientific community, with some advocating for the broader implementation of assisted migration despite these limitations, while others emphasize the importance of local adaptation, which may not keep pace with the rapid rate of climate change. This opinion paper proposes a novel pollen-based assisted migration strategy as a potential middle ground in this debate. By leveraging existing seed orchard infrastructure for controlled pollen transfer, this approach aims to enhance forest resilience through the introduction of genetic material from climatically suitable sources while acknowledging local adaptation. We assess the genetic implications of the proposed strategy through computer simulation. Additionally, we examine the ecological implications of assisted gene flow, discussing the potential benefits of heterosis and the risks of outbreeding depression in intra-specific hybrid populations. We further explore the advantages of pollen-based migration in mitigating phytosanitary risks, reducing economic barriers, and simplifying legal considerations compared to traditional seed or seedling transfer methods. Regional perspectives on adapting pollen-based assisted migration are provided, with specific examples from Northern and Central Europe. We highlight how this approach could be integrated into existing forestry practices and regulatory frameworks within the European Union. We conclude by advocating for the inclusion of pollen-based assisted migration in future international projects and operational forestry, emphasizing the need for adaptable policies that can support innovative forest management strategies in the face of climate change.

## 1 | Introduction

Forest ecosystems play a crucial role in mitigating climate change through climate regulation (Smith, Baker, and Spracklen 2023) and carbon sequestration, capable of removing billions of tons of carbon from the Earth's atmosphere (Canadell and Raupach 2008).

By fully utilizing available resources, knowledge, and technology, their potential for carbon sequestration could be significantly enhanced (IPCC 2022). However, tree species are particularly vulnerable to the adverse effects of climate change, such as rising temperatures and prolonged droughts, which increase their susceptibility to wildfires, diseases, and pests (Lindner et al. 2010).

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## RESPONSE OPEN ACCESS

## Alleviating the Concerns About Pollen-Based Assisted Migration

David Chludil<sup>1</sup> | Curt Almqvist<sup>2</sup> | Mats Berlin<sup>3</sup> | Arne Steffenrem<sup>1</sup> | Steven E. McKeand<sup>4</sup> | Jiří Korecký<sup>5</sup> | Jan Stejskal<sup>1</sup> | Jaroslav Čepík<sup>2</sup> | Fikret Isik<sup>4</sup> | Debojyoti Chakraborty<sup>3</sup> | Silvio Schueler<sup>6</sup> | Torsten Pook<sup>5</sup> | Christi Sagariya<sup>4</sup> | Milan Latibák<sup>1</sup>

<sup>1</sup>Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic | <sup>2</sup>Forestry Research Institute of Sweden (Skogforsk), Uppsala Science Park, Uppsala, Sweden | <sup>3</sup>Division of Forest and Forest Resources, Norwegian Institute of Bioeconomy Research (NIBIO), Stavanger, Norway | <sup>4</sup>NC State University Cooperative Tree Improvement Program, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, North Carolina, USA | <sup>5</sup>Department of Forest Growth, Silviculture and Genetics, Austrian Research Centre for Forests BFW, Vienna, Austria | <sup>6</sup>Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands

Correspondence: Milan Latibák (latibak@fid.czu.cz)

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We appreciate the opportunity to respond to the concerns of O'Neill et al. (2025) regarding our recent opinion paper in Global Change Biology (Chludil et al. 2025). There, we proposed a novel approach to assisted migration (AM) in forest trees, shifting the focus from the conventional translocation of seeds or seedlings to the transfer of pollen. Specifically, we suggested using seed orchards (SOs) to collect pollen from source populations and produce seeds in target regions.

To frame our pollen-based approach, we began by outlining major challenges commonly associated with AM, drawing on a comprehensive review of scientific literature. This broader context was essential for situating our proposal within diverse perspectives in the field. While we understand O'Neill et al.'s (2025) reservations about the relevance of specific challenges, we maintain that the arguments we cited reflect legitimate, widely discussed issues. Therefore, in this response, we focus specifically on their concerns regarding pollen-based AM.

The authors identify the necessity of SOs in both source and target regions as a potential disadvantage. However, SO networks are often dense and widely distributed. In the European Union and Great Britain alone, 1503 SOs represent 40 species and hybrids (Chludil et al. 2025; section 4 and Figure 2). In Canada, 249 tree breeding programs with associated SOs have recently

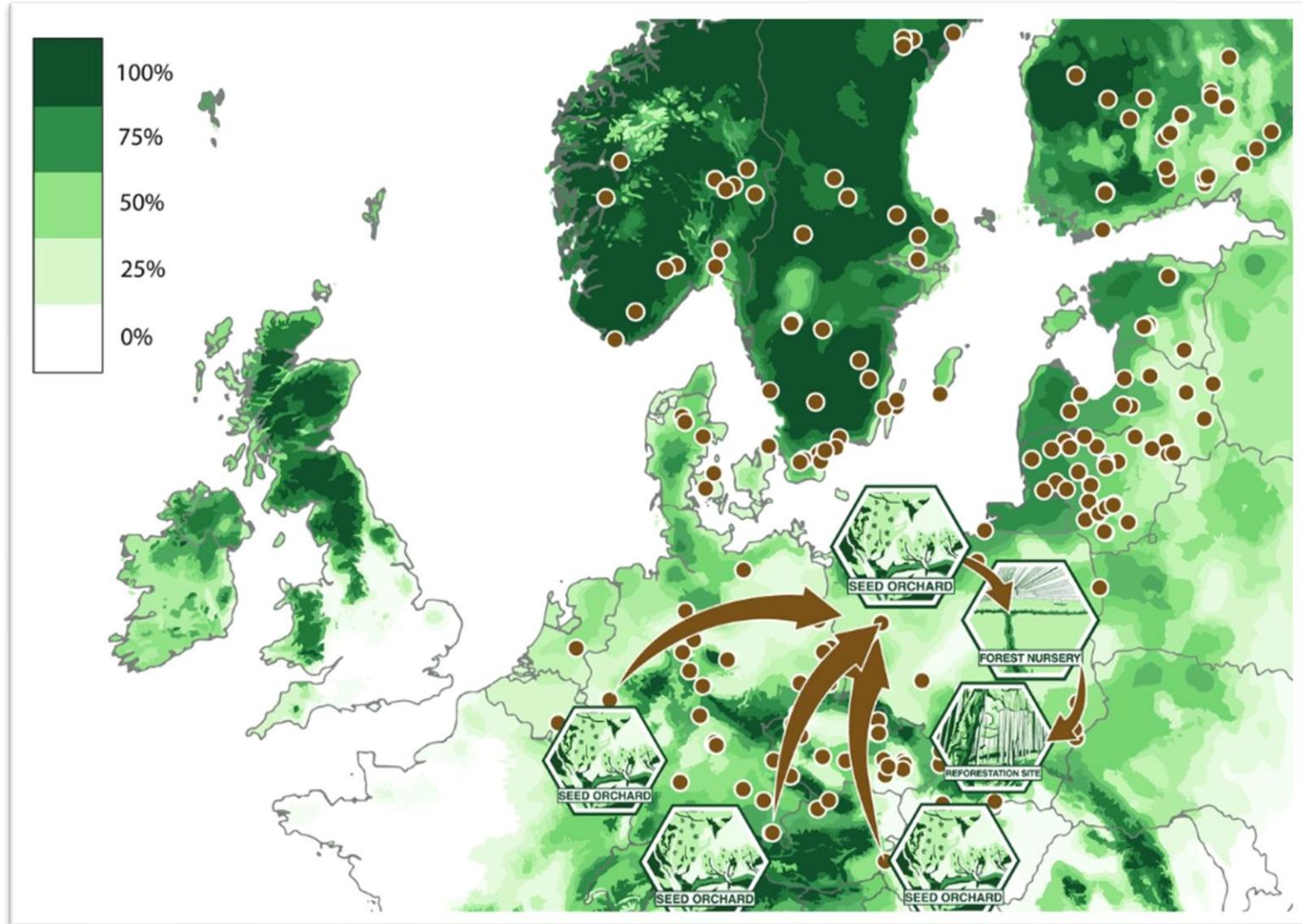
been documented, covering 25 different species (Thomas et al. 2024, Table 1). The SO network is particularly dense in British Columbia and Quebec. Reid (2008; Table 1) reported 113 SOs in British Columbia, while Culas and Bettez (2013) stated 89 in Quebec. We stress that a substantial SO network is equally important for seed collection under conventional AM, as SOs typically consist of genetically tested genotypes, unlike forest stands.

To address the second concern, we acknowledge that pollen handling and controlled pollination can pose operational challenges. However, many SOs globally are already equipped for pollen-based AM, with controlled mass pollination (CMP) or supplemental mass pollination (SMP) routinely applied (Figure 1). These practices demonstrate the feasibility of implementing similar approaches elsewhere.

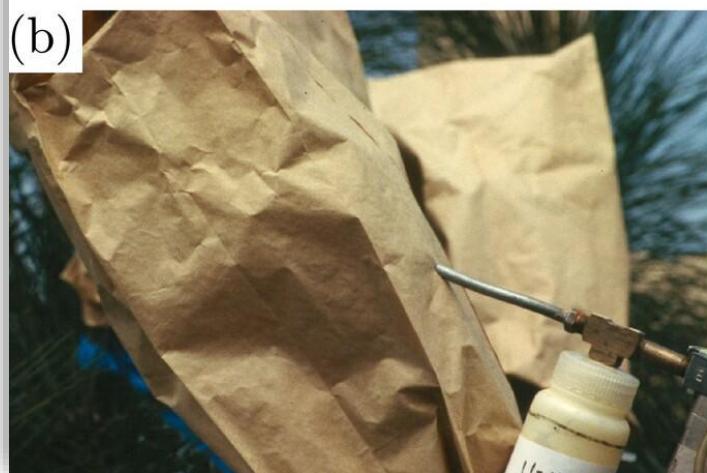
In response to the third concern regarding the potentially reduced efficiency of pollen-based AM, we contend that our simulations likely underestimate its potential adaptive response (Chludil et al. 2025; section 5). Due to a complex underlying genetic architecture, the adaptive response is not simply additive, even without heterosis. Moreover, as previously noted, our modeling did not incorporate several key benefits of pollen-based AM. For instance, pollen collection is unlikely to reduce

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## Current problems of assisted migration

## Solution by pollen transfer



### Uncertainty in new conditions

Both biotic and abiotic factors can limit plant establishment in new areas even if climate conditions are optimal.

Blooming phenology mismatch may limit the gene-flow between introduced and local population.

Hybrid progeny inherits traits close to the local optima due to the local and introduced gametes. The local parent is also responsible for epigenetic priming enhancing phenology overlaps with the local population.



### Phytosanitary risk

The translocation of seeds and seedlings can introduce non-native pests and pathogens.

Pollen transport significantly reduces the risk of pathogen transmission.



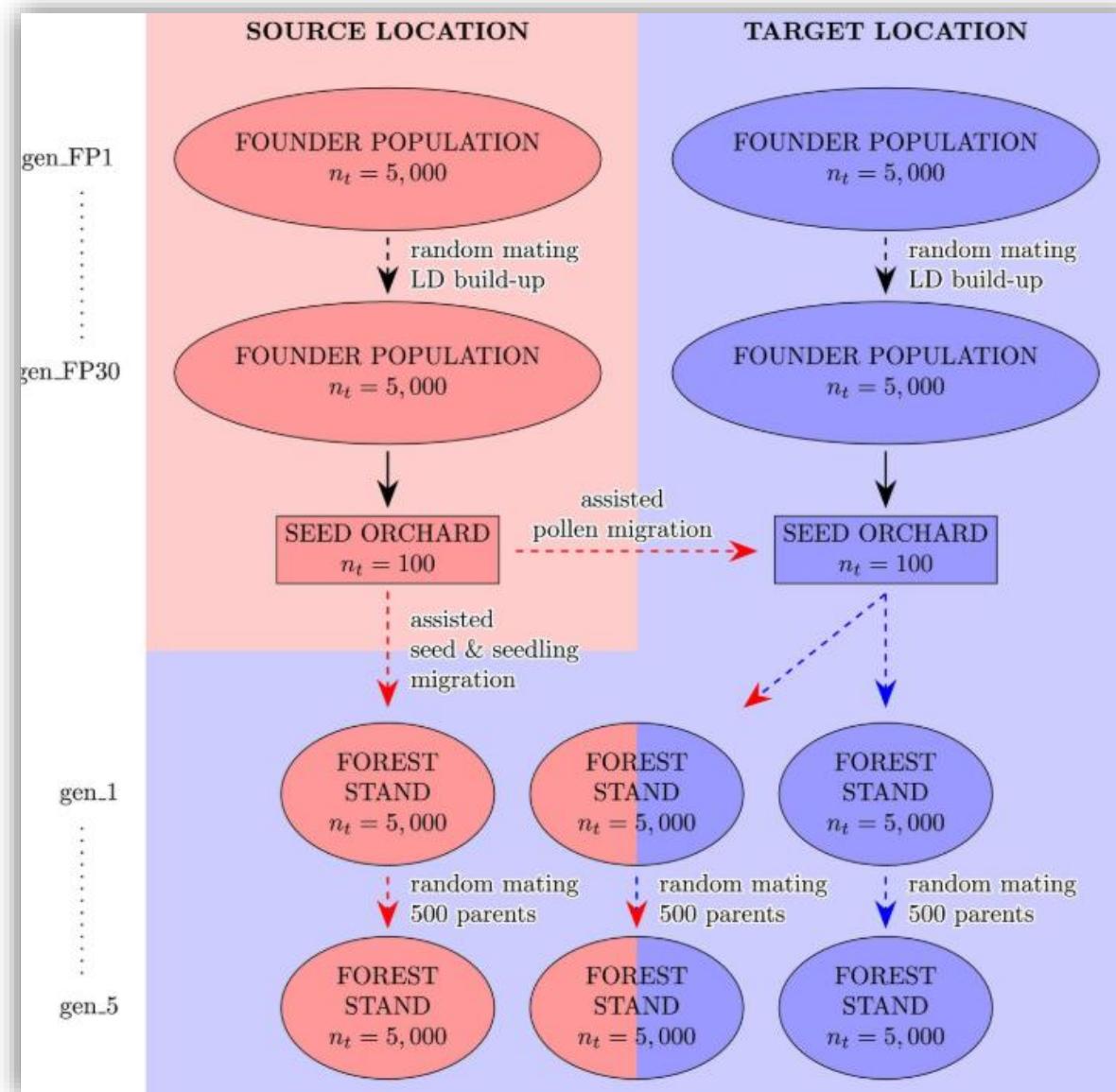
### Stakeholder struggles

Local nurseries face economic and knowledge barriers in adapting to new forestry practices required for assisted migration.

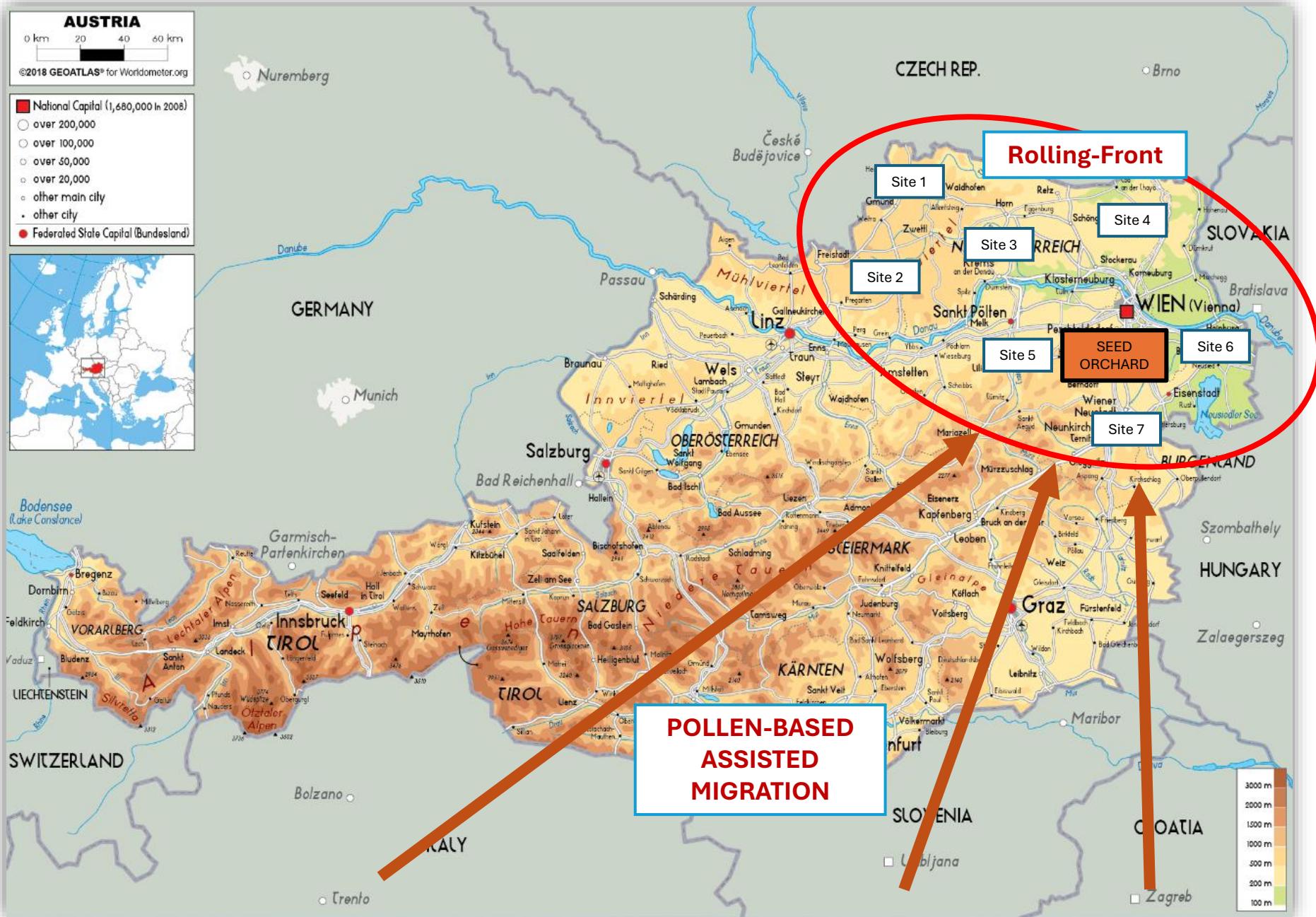
Traditional forestry practices often prioritize local provenance due to their historical balance of growth and hardiness.

There's no need for nurseries to take any action, as the downstream process of assisted pollination will result in business as usual.

Proposing a dynamic policy framework that maintains local adaptations and incorporates preadapted genotypes together with testing resulting hybrids through natural selection



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# Proposed RFLB+PAM system vs. Genomic Selection

**Fastest gain in today's conditions:** stable training set, short rotation → lean to GS, keep multi-environment training updated

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