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A Strategy for the Second Breeding Cycle of *Larix x marschlinsii* in Québec, Canada Including Experiments to Guide Interspecific Tree Breeding Programme

By M. PERRON¹⁾

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Abstract

A strategy for the genetic improvement of *Larix decidua* P. Mill., *L. kaempferi* (Lamb.) Carrière and their hybrid (*L. x marschlinsii* Coaz) in Québec, Canada, was set up to provide short and long-term genetic gains, as well as basic populations for some fundamental experiments. A reciprocal recurrent selection with forward selection strategy (RRS-FS) will be applied by using a pollen mix breeding with a partial population paternity analysis (PMX/WPPA). The genetic mechanisms responsible for heterosis among trees remain poorly understood. This complicates the implementation of multi-species genetic improvement programmes seeking to achieve the full benefits of interspecific hybridization. Various strategies to exploit heterosis are outlined. To expand our knowledge and guide our future choices, basic research has been integrated

directly into breeding and testing activities, as well as through experiments integrated within activities leading to recommendations about the top-ranked families to be used in reforestation (cuttings). These experiments can also serve as the basis for future studies seeking a better understanding of heterosis through molecular genetics.

Key words: paternity analysis, heterosis, interspecies hybrid, interspecific breeding strategy, polymix crossing, *Larix x eurolepis*.

Introduction

After more than 30 years of testing introduced larches, the Ministère des Ressources naturelles et de la Faune du Québec (MRNF-Q; Québec Ministry of Natural Resources and Wildlife) has passed an important milestone with respect to intensive silviculture in Québec. Effectively, we have decided to progress toward a second breeding cycle of *Larix x marschlinsii* (Coaz), notably because this larch variety has been identified as the most productive in Québec commercial forests (STIPANICIC, 1999). This variety of hybrid between European larch (EL;

¹⁾ MARTIN PERRON. Direction de la recherche forestière, ministère des Ressources naturelles et de la Faune du Québec (MRNF) 2700, rue Einstein, Québec (Québec), Canada G1P 3W8. Telephone: (418) 643-7994 #6547, Fax: (418) 643-2165. E-Mail: martin.perron@mrnf.gouv.qc.ca

Larix decidua Mill.) and Japanese larch (JL; *L. kaempferi* [Lamb.] Carrière) was first discovered in a nursery in Dunkeld, Scotland in the early 20th century (HENRY and FLOOD, 1919 cited in PÁQUES, 1989). Though other hybrid larch varieties exist, hereafter the designation 'hybrid larch or HL' will refer exclusively to hybrids between EL and JL, regardless of cross direction, e.g., which parent is male or female (e.g. TOUSIGNANT and STIPANICIC, 2000). Otherwise, HL will be referred to with the maternal parent listed first, e.g., E x J or J x E. The main interest of using HL for applied silviculture resides in its improved growth rate compared to its parents. For example, FERRAND and BASTIEN (1985) reported that the mean annual increment (MAI) of HL showed heterobeltosis: 26 years after planting, the hybrid's MAI was 16.7 m³ ha⁻¹ yr⁻¹, whereas that of the JL was only 12.8 m³ ha⁻¹ yr⁻¹. The terms heterosis and heterobeltosis are used respectively to describe an H₁ hybrid's superiority relative to its parents' mean value and that of its best individual parent with respect to a given trait. *L. x marschlinii* also has other interesting qualities, particularly with respect to its wood properties and disease resistance (e.g. ROBBINS, 1985). A study comparing the wood properties of 15-year old HL to that of its parents grown at the same location for the same period of time (DERET and KELLER, 1979 cited in BASTIEN and KELLER, 1980), showed that the hybrid's wood (i) was more heterogeneous (greater variation in within-ring density), (ii) had a volumetric retractability (9.4%) intermediate between those of its parents (8.2 and 9.6%), and (iii) presented better mechanical properties. In Europe, *L. decidua* is a widespread but fragmentarily distributed mountain species (e.g. VIDAKOVIĆ, 1991; BERGÈS and CHEVALIER, 2001). It is valued for the properties of its wood and its resistance to freezing (e.g. BASTIEN and KELLER, 1980; FOWLER et al., 1988). On the other hand, given that *L. kaempferi*'s natural distribution is limited to a few thousand hectares on Japan's Hondo Island (e.g. BERGÈS and CHEVALIER, 2001), it is only found on well drained mountain soils located between 600 and 2500 m in elevation (e.g. VIDAKOVIĆ, 1991). Its sturdiness of growth and its resistance to European larch canker (*Lachnellula willkommii*) are the main traits it should pass on to hybrid varieties (e.g., FOWLER et al., 1988; BASTIEN and KELLER, 1980). It is also more resistant to needle rust (*Mycosphaerella laricina*) than is *L. decidua* (e.g. OSTRY et al., 1991).

The use of interspecific hybridization in forest tree breeding programmes has garnered increasing interest, particularly among genera such as *Pinus* (e.g. DIETERS et al., 1997; BRAWNER et al., 2005); *Populus* (e.g. LI and WU, 1996); *Eucalyptus* (e.g. POTTS and DUNGEY, 2004) and *Larix* (e.g. PÁQUES, 1989; LI and WYCKOFF, 1994). This interest rests upon three main points (e.g. ZOBEL and TALBERT, 1991): (i) heterosis and heterobeltosis of a given trait, (ii) the combination of favorable traits leading to a greater adaptability, and (iii) the transfer of a desirable trait of one of the parental species. Compared to breeding through traditional intraspecific crosses, improving varieties through interspecific hybridization is subject to a number of technical constraints: (i) the need to maintain a breeding population of the two parental species represents significant costs, (ii) asynchrony in the flowering seasons of the parental species (e.g. *Larix decidua* x *L. kaempferi*), results in a greater difficulty in generating interspecific (vs. intraspecific) hybrid seed (e.g. PHILIPPE and BALDET, 1992; BONNET-MASIMBERT et al., 1998), and (iii) instances of full emergence of interspecific-cross-generated seed are much lower (e.g. ARNOLD, 1997; TRÖBER and HAASEMANN, 2000). Pre- and post-zygotic factors which reduce the yield of interspecific hybrids were reviewed by ARNOLD (1997). Even though basic hypotheses regarding heterosis were

enunciated in the early 20th century, some debate remains within the scientific community as to its causes. One hypothesis is that it results from a dominance-type genetic effect. This implies that the resultant superiority arises from the elimination of one of the parent species' deleterious alleles (e.g. DAVENPORT, 1908; BRUCE, 1910; KEEBLE and PELLEW, 1910; JONES, 1917). On the other hand, the overdominance hypothesis postulates that heterosis results from heterozygosity at a given locus being superior to any combination of homozygous alleles at the same locus (e.g. SHULL, 1908; EAST, 1936). The present failure to understand the genetic underpinnings of interspecific heterosis in plants complicates the choice of a breeding strategy for advanced-generation breeding in forest trees.

In this paper we present an efficient, cost-effective breeding strategy for the second breeding cycle of *Larix x marschlinii* by the MRNF-Q, a brief overview of various strategies that are recommended for the improvement of the interspecific hybrid, of the integration of fundamental research to adequately mold the breeding strategies to be employed in our introduced larches breeding programme's third cycle, as well as to guide other interspecific tree breeding programmes.

MRNF-Q breeding strategy for the second breeding cycle of *Larix x marschlinii*

Given the recent (2001) rise in demand for HL by the Québec forest industry, but prior to the establishment of the second-generation, the strategy of our introduced larch species breeding programme was revised and corrected. The improvement programme for an interspecific hybrid variety is necessarily more exacting than that for a single species, which limits the number of possible areas of improvement for a given investment. We currently know very little regarding the plasticity of larches introduced into Québec. Only a small portion of genetic entries (provenances or progenies) have been tested over a large range of latitudes. However, they appear to adapt well to conditions prevailing between latitudes of 45° 41' N to 48° 21' N, since the top-ranked genetic entries perform in the same manner from one site to another (7 sites) (STIPANICIC, 1999). Consequently, only a single breeding zone will be used during the programme's second cycle. The next breeding cycle will be considered as our second, though this is not entirely true, since a portion of the EL parents have been selected from a second-generation. Indeed, when EL from the oldest experimental plots reached their sexual maturity in the early 1980s, seeds were harvested and two half-sib progeny tests were undertaken. These offspring represent a second-generation even if the breeding groups were not structured. Furthermore, with respect to the parent material they represent a partial generation (or an incomplete evaluation population) since the selection of parents did not entail an overall analysis or sampling of available materials from which to make such a selection. The present breeding cycle will no doubt profit from a portion of its parents originating from this more advanced-generation. This situation is not exclusive to our programme; other breeding populations have been generated from parents having different levels of domestication (e.g. BEAULIEU, 1996).

The principal objective of the MRNF-Q's introduced larches breeding programme is to increase *L. x marschlinii*'s yield of high quality wood. At present, the programme involves the use of two parental species and the release of new hybrid varieties. Secondary objectives include: (i) identifying hybrid families best suited to vegetative propagation (cuttings and somatic embryogenesis), (ii) choosing a breeding strategy for the next generation, (iii) recommending parents to generate upcoming

production populations, and lastly (iv) identifying breeding populations to generate the third-generation.

For the 2003 selections, three main classes of comparative plantations were once more analysed: (i) interspecific provenance and half-sib progeny tests (analysed by species and genetic entry), (ii) provenance tests, and (iii) half-sib progeny tests. Given their high mortality rate or their poor performance, some candidate genetic entries were stricken from the new analyses (rejected without further analysis). These plots were located in arboretums where other better performing test plots were present nearby. Comparative plantations made up exclusively of HL were also set aside. A total of 26 comparative plantations (over 40 000 seedlings planted) served in the selection of half-sib families (HSF) or provenances, while tree selection occurred on twelve among them. As much as possible, selections were made in more than one bioclimatic domain. According to the data available, progeny and provenance selection was initially based on an overall height after 9, 10 or 15 years. Thereafter, a mass selection based on trunk straightness, crown quality (branch size, angle and number) and disease resistance (absence of damage) criteria was undertaken. In order to facilitate fieldwork, candidate-trees were preselected using results of the previous measurement of trunk straightness and total height. Selected trees (ST) had to have a height to diameter ratio (cm cm^{-1}) inferior to one hundred. HL were excluded from the ST by using a molecular markers validation procedure (not shown) (e.g. ACHÉRÉ et al., 2004; GROS-LOUIS et al., 2005). Finally, with the exception of four provenances of JL not represented by HSF (two with five STs and two with six STs), a maximum of four STs were drawn from each genetic entry, resulting in a total of 80 *L. decidua* and 80 *L. kaempferi* STs being retained. Based on theoretical estimates, NAMKOONG et al. (1980) concluded that advanced breeding programmes for allogamous tree species required a breeding population of at least 50 different parents to prevent the risk of consanguinity and the loss of desirable alleles. The parents which are to make up the MRNF-Q programme's second-generation breeding populations are drawn from a limited gene pool (280 EL and 137 JL genetic entries) when compared to our native species. This situation can be explained by our difficulty in obtaining basic breeding materials and the limited funding granted to such a programme. For both parental species, a large portion of genetic entries were tested only on one site. Therefore, estimation of unbiased heritability and an unbiased estimate of genetic gain are not available for a large portion of our candidate genetic entries. In addition, variability of estimated heritability is very large, because some tests (notably interspecific half-sib progeny tests) were based only on few genetic entries with very large differences between them. For example, biased individual tree narrow-sense heritability of 15-year total height in EL ranged between $0,27 \pm 0,18$ and $1,34 \pm 0,81$, depending on the site (PERRON, unpublished). The ST for EL were drawn from 42 HSFs (15% of tested genetic entry), representing natural stands and secondary sources (for details see Annex I). A large portion of the JL breeding population was drawn from 13 provenances (natural, seed orchard or plantation seed lots) and the remaining JL were drawn from 12 HSFs (for details see Annex I). In this case the retained genetic entries (prov. and HSF) represented 18% of those tested.

Our new strategy for HL takes into account the programme's material and knowledge assets, present and anticipated needs of the Québec forest industry, the latest developments in the field of genetics and the exotic status of this variety. Given that the reciprocal recurrent selection with forward selection (RRS-FS) strategy is among the safest for interspecific breeding

strategy (KERR et al., 2004b), it will be implemented for the second-generation. Under this strategy, every tree of both parental species' breeding populations (in our case EL and JL) is crossed in such a manner that both intraspecific HSF and interspecific HSF are produced. Selection is then based on an index drawn from general combining ability (GCA) and general hybridizing ability (GHA) values. This will also guarantee a greater domestication of parental species, an element not to be neglected at this stage of our programme. For each taxa (EL, JL, E x J and J x E), breeding and tests implemented with RRS-FS will allow a good estimation of genetic parameters such as heritability, GCA, genetic correlations among traits and genotype-environment interaction (G x E), as well as an estimation of the correlation between GCA and GHA. This correlation is an important element in determining the best hybrid breeding strategy (KERR et al., 2004a). Also, the third breeding cycle of introduced larches in Québec will undoubtedly benefit from such accurate genetic parameter estimates. The exotic status of HL in Québec and the RRS-FS strategy dictate the implementation of a simple and low-cost mating design, so we will combine pollen mix breeding with a partial population paternity analysis (PMX/WPPA) (LAMBETH et al., 2001). This implies that provisional candidates of the next breeding population will be genotyped and paternity will be assigned before the final selection of the breeding population. This corresponds to the simplest scenario they recommended. Final selection should be based on fathers, GCA, GHA or both (according to selection strategy retained for the third breeding cycle) and an acceptable level of relatedness such as group-merit (e.g. LINDGREN and MULLIN, 1997; ROSVALL and ANDERSSON, 1999). A similar strategy using open-pollinated and parental identity for forward selection was adopted recently for a minor introduced species in New Zealand: *Eucalyptus nitens* (GEA et al., 2007). It will also achieve most of the general objectives of a mating design such as: estimation of the breeding value, production of the recruitment population, control of the level of inbreeding and accurate estimation of genetic parameters. Furthermore, the needs of our production population are mainly based on individuals with good GHA. Following procedures recently developed by the MRNF-Q, HL seeds are produced in an indoor orchard using directed mass pollination (electrostatic gun) and pot-grown ramets with a pollen mix from a large number of trees (COLAS et al., 2008).

According to the principles of RRS-SF and PMX/WPPA, two parental species' PMX will be used to evaluate the GCA and GHA of each species' breeding population. To complete controlled crosses in a reasonably short period of time and control kinship, no more than one EL ST per HSF will be retained for the EL PMX (max. of 42 ST). Collecting enough pollen for all ST in the breeding population could greatly delay the beginning of crosses, as usually young grafts produces more female flowers. For the same reason, no more than two JL STs per provenance and one JL ST per HSF should be retained to form the JL PMX (max. of 38 ST). Thus 160 HL HSFs (80 E x J and 80 J x E) will be generated, as well as 80 HSFs of each parental species, for a total of 320 HSFs. When associated with multi-site testing, such a mating design allows the identification of individuals best suited for open pollinated seed orchards or mass pollination in indoor orchards, and provides plants for reforestation which are adapted to a wide range of bioclimatic conditions. Ideally, the eight sites chosen for the evaluation of GCA and GHA, will be located in three different bioclimatic regions, ranging from a sugar maple-yellow birch stand to a balsam fir-white birch stand (i.e., from roughly 46° to 49° N). At each site, a progeny test will be set up in a two-stage nested

randomized complete block design. The first stage will be taxa (EL, JL, E x J and J x E), randomized for each block (total of 16 blocks). The second stage will be for each of the 80 HSFs per taxa (total of 320 HSFs) randomized within taxa and blocks with single-tree plots. Consequently, all HSFs will be represented by 16 trees per site, for a total of 128. This number of trees per HSF follows the general recommendation of 10 to 20 individuals per family at each site when testing multiple sites (COTTERILL and JAMES, 1984). This experimental design of grouping all taxa on several sites, has been selected because it is already known that these taxa will greatly vary in growth. Such a design will enable an accurate estimation of the GCA and GHA of the 160 parental genotypes, as well as estimating G x E. In addition, this experimental design will appropriately control environmental variation so as to allow one to 1) compare the E x J and J x E performance; 2) estimate the level of heterosis ($HV = \mu_{E \times J}$ [or $\mu_{J \times E}$] - $1/2 (\mu_E + \mu_J)$ / $1/2 (\mu_E + \mu_J) * 100$); and 3) estimate the correlation between GCA and GHA. This series of tests will also be useful in deciding whether more than one breeding zone is required during the subsequent breeding cycles. For the production population, the HSFs best suited to Québec's diverse bioclimatic conditions will be identified. Ten-

year measurements (around 2025) will provide information necessary to the selection of parents for the third-generation. For some of the top-ranked hybrid families, wood quality parameters will be measured and considered during the subsequent selection process. Eight elite trees (ET) per species (8/80= 10%) will be recommended for indoor orchards. Genotyping and paternity analysis will be conducted only after the next cycle's breeding strategy will have been selected. The major activities for the second breeding cycle of *Larix x marschlinsii* are presented in *Table 1*.

We expect our RRS-FS with PMX/WPPA strategy to generate 320 HSFs to be evaluated and form the recruitment population for the third-generation. In comparison, the strategy of LI and WYCKOFF (1994) planned to generate 700 families (150 *L. x marschlinsii*, 400 *L. decidua* and 150 *L. kaempferi*). Finally, for the 2nd breeding cycle of *L. x marschlinsii*, resource requirements will represent approximately 38–75% fewer crosses and 50–65% fewer planted trees, as compared to our three major breeding programmes (*Table 2*).

Lastly, the intensity of selection was greater in the 2nd generation production population: 20 EL and 20 JL, selected among

Table 1. – Idealized schedule for the second breeding cycle of *Larix x marschlinsii* and integrated fundamental experiments.

Cycle year	Year	<i>L. x marschlinsii</i> breeding strategy (RRS-FS)	Fundamental experiments
0	2003	Select 130 EL—160 JL and validate taxa with molecular markers.	
1-6	2004-09	Retain and graft 80 EL and 80 JL (BP).	Experiment 1. Heterosis Select 10 trees per parental species among the best phenotypes of each BP. Conduct control pollinated mating design (see <i>Table 3</i> ; 60% done in 2007).
9	2012		Experiment 1. Heterosis Plant 2 full-sib progeny tests.
5-10	2008-13	Perform intra and interspecies PMX cross for the 160 genotypes.	
13	2016	Plant 8 PMX tests, per parental species and per hybrid type, to estimate GCA and GHA of the 160 genotypes.	Experiment 2. E x J or J x E hybrid type Set up the PMX tests of the breeding strategy in a two-stage nested randomized complete block design with single-tree plots.
14	2017		Experiment 3. SYN vs RRS-SF strategies Select and graft 36 genotypes per taxa among the best phenotypes from <i>Experiment 1</i> .
17-21	2020-24		Experiment 4. GCA and GHA based selection strategies For EL and JL, select 3 x 12 elite-trees based on 3 selection strategies: AGC, AGH and AGC + AGH. Perform 2 interspecies PMX cross for each selection strategy.
19-22	2022-25		Experiment 3. SYN vs RRS-SF strategies Conduct 2 interspecies and 2 inter hybrid types PMX cross.
23	2026	Select and graft superior individuals from EL half-sib families that have highest breeding values based on: 1) GCA, 2) GHA and 3) GCA + GHA. <i>idem</i> for JL and HL, but based on GHA only.	

BP = breeding population; **EL** = European larch; **E x J** = hybrid with an EL mother; **GCA** = general combining ability; **GHA** = general hybridizing ability; **HL** = hybrid larch; **JL** = Japanese larch; **J x E** = hybrid with a JL mother; **PMX** = pollen-mix; **RRS-SF** = reciprocal recurrent selection with forward selection; **SYN** = synthetic species.

Table 2. – Comparison of planned breeding, testing efforts and establishment of recruitment populations for the second breeding cycle of *Larix x marschlinii* within our three major breeding programmes (also 2nd cycle).

Taxa	Breeding pop.	Progeny test	Recruitment population
No. of breeding zones ¹⁾	No. of parents	No. of progenies/ No. of HSF ^{3)/} No. of tests	No. of progenies/ No. of FSF ^{4)/} No. of tests
Exotic larches ²⁾ (1)	160	≈ 40 900/ 320 HSF/ 8	directly in HSF progeny tests
<i>Picea glauca</i> (2)	240	≈ 25 900/ 240 HSF/ 2 x 5	≈ 56 900/ 272 FSF / 2 x 5
<i>Picea mariana</i> (3)	3 X 300	≈ 72 000/ 3 x 300 HSF/ 10	≈ 48 000/ 2 X 200 FSF / 10
<i>Pinus banksiana</i> (2)	2 X 300	≈ 57 600/ 2 x 300 HSF/ 2 x 4	≈ 38 400/ 2 X 200 FSF/ 2 x 4

¹⁾ Number of breeding zones with activities for the second breeding cycle.

²⁾ Data computed for European larch, Japanese larch and hybrid larch (excluding fundamental research).

³⁾ HSF = half-sib family.

⁴⁾ FSF = full-sib family.

the best phenotypes of each species, will be used as parents, compared to 80 STs per species in the breeding populations. In order to limit the kinship ties and to maintain genetic diversity, a maximum of two STs have been used per genetic entry. The EL selected represents sixteen HSFs from nine provenances, whereas the JL represents eight provenances and six HSFs.

Overview of various interspecific breeding strategies

The principal breeding strategies recommended for the improvement of interspecific hybrids are based on various genetic effects. The pros and cons of these strategies are presented below. The first and simplest strategy, in accordance with KERR et al. (2004b) will be termed the Synthetic Species Strategy (SYN). It involves the use of H₁ hybrids as genitors of the next generations (H₂, H₃, etc.). This strategy's main advantage is that, in the long-term, it eliminates the need to maintaining populations of pure species to generate H₁ offspring, and problems associated with this mode of production. It is employed when hybrid vigour is under additive gene control or when a combination of traits from both species is desired (e.g. NAMKOONG et al., 1988). A study of the influence of parent species EL and JL on a number of desirable traits in their H₁ progeny (*L. x marschlinii*) noted that 11-year tree height, 15-year tree-volume, 6-year branch angle and 13-year wood density were under additive gene control (PÂQUES, 2004). In contrast, other traits (proportion of heartwood and modulus of elasticity) were under non-additive gene control. Nonetheless, the gene control of still other traits (e.g., trunk straightness) varied between two factorial mating designs (12 x 6 vs. 9 x 7, with nine female EL in common). This is why PÂQUES (2004) stated that in order to pin down the genes controlling *L. marschlinii*'s traits of interest, a greater number of crosses would be necessary. In an experiment on vegetative propagation, PÂQUES (1992) noted that 8-year tree-heights of H₂ lines were intermediate between those of H₁ lines and *L. kaempferi*. LI and WYCKOFF (1994) cited unpublished data indicating a slightly greater variability in their H₂ lines, but no loss in vigour. Greater variability is undesirable, particularly for traits where uniformity is an important aspect related to end product quality.

The second strategy, is based on a recurrent selection for GCA in the pure species (PSS), as defined by KERR et al. (2004b). Under this PSS strategy, elite trees (ET) of each species are used to generate the hybrid variety destined for afforestation. Given that most traits were under additive gene

control, and that, compared to other breeding strategies, costs and duration of one round of breeding were lesser, NIKLES (1992 cited in KERR et al., 2004a), concluded that a PSS strategy was the most effective.

Under the third strategy, reciprocal recurrent selection (RRS), one cycle of improvement lasts two generations: (i) a first generation made up of H₁ progenies is tested, and ET are selected on the basis of their GHA; (ii) a second-generation, made up of two intraspecific populations, is evaluated through comparative plantations, followed by a GCA-based selection. This strategy was developed to exploit traits under both additive and simple dominance gene control [COMSTOCK et al. (1949) cited in NAMKOONG et al. (1988)]. In 1972, upon review of a number of experiments on animals, BELL (1972, cited in KERR et al., 2004b) deduced that RRS was likely the best strategy to use when complete dominance or overdominance gene control were at work. A few years later, EBERHART (1977 cited in KERR et al., 2004b) drew the same conclusion with respect to corn (*Zea mays* L.) hybrids.

The fourth strategy, reciprocal recurrent selection with forward selection (RRS-FS), derives from RRS, and was created to reduce the duration of the breeding cycle. This requires that GCA- and GHA-based crosses all be undertaken and tested at the onset of the cycle. Selection is then based on an index drawn from GCA and GHA values, along with measured parameters of individual candidate-trees. However, within this strategy's framework, breeding populations can be selected according to a number of criteria: GCA, GHA, or both. Here too, the type of gene control at work is important. WEI and VAN DER WERF (1994) showed that, given a moderate heritability (0.2), the correlation between the performance of hybrids and intraspecific lines was roughly 1.0, and that H₁ progenies' genetic response was maximized by a selection based exclusively on GCA. Based on computer simulations which indicated that RRS-FS and RRS strategies presented consistent annual genetic gains over a much wider array of genetic structures than either PSS or SYN, KERR et al. (2004a) concluded that the risks entailed by the former strategies were less than those for the latter strategies. Even though they implemented a SYN strategy, LI and WYCKOFF (1994) suggested the maintenance of pure species and evaluated hybrid populations for (i) greater security, and (ii) the ability to react quickly when more information becomes available. This suggestion's resource requirements fall midway between the SYN and RRS-FS strategies; there are fewer crosses to make and evaluate, but stands of pure species populations must be maintained.

Fundamental research integrated into the MRNF-Q's second breeding cycle of *L. x marschlinsii*

In this context, our programme, in seeking to acquire basic knowledge critical to the long-term pursuit of breeding activities, undertakes to (i) understand the genetic causes of juvenile heterosis in HL, (ii) establish whether the offspring of reciprocal crosses (i.e., E x J vs. J x E) perform differently, (iii) estimate differences between SYN and RRS-FS strategies' effectiveness, and (iv) test the relative effectiveness of selection strategies based on GCA vs. GHA with ET, in order to simplify both the overall programme and the generation of hybrid varieties, over the long-term. Therefore, four fundamental experiments will be integrated into the second breeding cycle of HL and the creation of families that serve to recommend the best hybrid HSFs for production. This will allow the acquisition of the genetic database required for the development of a more effective and economically feasible hybrid breeding strategy for the third-generation. The work under this empirical approach will certainly be long and costly compared to computer simulations, but will undoubtedly indicate the right approach to take. This material can also serve as the basis for other studies, e.g., molecular genetics, tree physiology, wood science, since it will already be laid out in known designs, and replicated in several bioclimatic regions. NAMKOONG et al. (1988) stated that a better understanding of a species' genetic structure is useful in targeted breeding, not only in knowing in hindsight the sources of variation and in optimising the sampling of these, but also in taking advantage of its structure and anticipating the development of multiple varieties.

Experiment 1.

Origin of heterosis in L. x marschlinsii: breeding (≈ 2005–2009) and testing (≈ 2012–2021)

A better knowledge of the genetic causes of heterosis will help us to select our breeding strategy for the third breeding cycle of introduced larches. The ten best phenotypes of each species will make up half of the second-generation production population and will be part of the second-generation EL and JL breeding populations. In addition, they will be used for the first fundamental experiment integrated in the planned crosses des-

igned to provide recommendations for the propagation by cuttings of the best HL full-sib families throughout the second breeding cycle (Table 1). As suggested by LI and WU (1996), intra- and interspecific factorial crosses will be organised into four 5 x 5 subgroups (Table 3). This factorial mating design (60% complete in 2007) will itself generate four taxa (EL, JL, E x J and J x E): 50 HL full-sib families (25 E x J and 25 J x E), and 50 full-sib intraspecific families (25 EL and 25 JL). This experiment seeks mainly to: (i) understand the genetic causes of juvenile heterosis in HL, and (ii) recommend the best families for propagation by cuttings. In addition, a preliminary estimate of correlations between GCA and GHA can be achieved with the few parents involved. Finally, this experiment will contribute toward a future fundamental experiment comparing SYN and RRS-FS (Experiment 3 and Table 1). This cross design, requiring few genitors, will be conducted within the first generation's evaluation plantations, thus reducing the waiting time for the sexual maturation of grafts. Two comparative plantations will be set up in two contrasting bioclimatic regions of southern Québec, namely in sugar maple-basswood (±46°N) and balsam fir-yellow birch (±49°N) stands. A two-stage nested randomized complete block design will be set up, for similar reasons as described previously for our 2nd breeding cycle of HL. Again, the first stage will be taxa, randomized within each block (total of 25). The second stage will be for the 25 full-sib families per taxa (total of 100), that will be randomized within taxa and blocks as single-tree plots. Accordingly, all full-sib families will be represented by 25 trees per site, for a total of 50. Following five-year and ten-year measurements, the 10 top-ranked HL full-sib families out of 50 (20%) will be selected for propagation by cuttings. During the second season of nursery growth, height growth and some phenological traits will be measured to document the impact of annual growth variation on heterosis. Furthermore, we plan to measure the total height after planting (Year-one) and second-whorl branch lengths to evaluate correlations between these traits and Year-five total height. Indeed, a number of authors have observed a link between crown width and larch vigour (e.g. LACAIZE and BIROT, 1974; STIPANICIC, 1984; BALTUNIS and GREENWOOD, 1996).

Table 3. – Intra and interspecific 10 x 10 factorial mating design in sub-groups.

Parent ♂		EL ₁	EL ₂	EL ₃	EL ₄	EL ₅		JL ₁	JL ₂	JL ₃	JL ₄	JL ₅	
Parent ♀	EL ₆	X	X	X	X	X		X	X	X	X	X	
	EL ₇	X	X	X	X	X		X	X	X	X	X	
	EL ₈	X	X	X	X	X		X	X	X	X	X	
	EL ₉	X	X	X	X	X		X	X	X	X	X	
	EL ₁₀	X	X	X	X	X		X	X	X	X	X	
	JL ₆	X	X	X	X	X		X	X	X	X	X	
	JL ₇	X	X	X	X	X		X	X	X	X	X	
	JL ₈	X	X	X	X	X		X	X	X	X	X	
	JL ₉	X	X	X	X	X		X	X	X	X	X	
JL ₁₀	X	X	X	X	X		X	X	X	X	X		

EL = European larch; JL = Japanese larch.

Experiment 2.

L. decidua or *L. kaempferi* as mother trees of HL: breeding ($\approx 2008-2013$) and testing ($\approx 2016-2035$)

At present, we don't know if one hybrid type (E x J or J x E) outperforms the other in growth rate, because few progenies have been compared and few studies have used the same parent material. (e.g. PÁQUES, 1989; BALTUNIS et al., 1998). Documenting this question will help to determine the breeding strategy for the third breeding cycle, as well as the best way to produce our exotic larch for intensive silviculture in Québec. As seen before, this fundamental experiment is directly integrated in our second breeding cycle of HL. Recall that this was one of the reasons for using RRS-FS with PMX/WPPA crosses and the two-stage nested randomized complete block design. We will compare only E x J and J x E HSFs that have both mother and father used in the crosses. Therefore, we will compare approximately 40 HSFs per hybrid type. Since progeny tests will be established at eight sites, it will also be possible to evaluate whether hybrid types perform differently in contrasting bioclimatic conditions.

Experiment 3.

Comparing SYN and RRS-SF strategies: breeding ($\approx 2022-2025$) and testing ($\approx 2028-2034$)

The objective of this fundamental experiment is to evaluate the relative effectiveness of SYN and RRS-FS strategies, in order to simplify both the breeding cycle and seed production over the long-term. This will also generate third-generation HSFs that could rapidly be (as compared to the complete breeding cycle) recommended for the production population. The parents used to compare the effectiveness of the SYN strategy to the RRS-FS strategy will be selected in two stages. First, from the Year-5 results of Experiment 1 (Table 1) for each taxa (EL, JL, E x J and J x E), the three best phenotypes of the 12 best full-sib families out of 25, will be chosen as ST. To facilitate and accelerate the controlled crosses needed to compare the two breeding strategies, these ST will be grafted. Indeed, it is easier to complete controlled crosses indoors with potted grafts than with trees located in progeny tests. In addition, the cultural approach developed in Québec (COLAS et al., 2008) favors the flowering of larch grafts. Later, based on Year-10 results of Experiment 1 (Table 1), 64 ST will be chosen among 144 five-year ST. Two ST will be chosen per family among the eight Year-10 top-ranked full-sib families of each taxa: 32 H₁ (16 E x J and 16 J x E) and 16 ST per parental species. The HL family selection will be GHA-based, while that of pure species will be GCA-based. For this experiment, crosses will be set up in two interspecies PMX with 16 ST per species, to create 32 HL HSFs (16 E x J and 16 J x E). Two other inter hybrid type PMX with 16 ST per hybrid type will create 32 H₂ HSFs.

Experiment 4.

GCA and GHA-based selection strategies: breeding ($\approx 2021-2024$) and testing ($\approx 2027-2033$)

The objective of this study is to assess the effectiveness of the various selection procedures within the RRS-FS strategy, while supplying a potential of 72 new HL HSFs suitable for vegetative propagation. A backward selection of 36 elite-trees (ET) per parental species (EL and JL) will be based on Year-5 results from the series of tests on GCA and GHA (Table 1) and the use of best linear unbiased prediction (BLUP). Selecting ET only five years after planting may appear relatively precocious, but LI and WYCKOFF (1994) planned to generate their breeding populations of larches, from one generation to the next, at this young age. From these trees, 12 ET per species will be selected

according to their GCA, while another 12 ET per species will be selected according to their GHA. A further 12 ET per species will be selected according to an index derived from both the GCA and GHA. Some trees will be selected by more than one selection process resulting in less than 36 ET per species. Six PMX crosses will be conducted to produce 12 E x J and 12 J x E HSFs for each selection strategy, for a total of 72 HL HSFs.

Experiments 3-4

Around 2027, for both experiments, two comparative plantations will be set up, in contrasting bioclimatic domains. The ten full-sib families recommended for vegetative propagation after Year-10 results of Experiment 1 will serve as controls. These complementary studies will provide information necessary to choosing breeding and selection strategies for the programme's third-generation, as well as the next mode of production. In addition, these experiments will generate new families of HL for the production of cuttings (e.g., advanced second-generation and third-generation). Measurements are planned at 5, and 7 years in order to (i) check how the genetic effects of crosses vary according to age (e.g. PÁQUES, 1992), (ii) allow the final selection of third-generation breeding populations, and (iii) make recommendations for the production of cuttings.

Long-term follow-up

Breeding population

Around 2026, a preselection of STs will be undertaken to form the third breeding populations. A maximum of 480 EL, will be preselected through GCA- and GHA-based selection using BLUP. We plan to select EL trees as follows: four ST per HSF in the 40 top-ranked EL HSF based on GCA, the same selection scheme based on GHA, and based on an index derived from both GCA and GHA. We will undoubtedly have fewer than 480 EL STs as some HSFs will be retained by more than one of these three selection strategy. For JL and both hybrid types, we will have a maximum of 160 preselected trees per taxa. In effect, we plan to select trees with the same number of ST and HSF as for EL, but based only on GHA. Thus, for each type of selection and taxa, the final selection intensity will be 2.502 (1.6% of candidate trees or 80 ST from 5120 candidate trees [128 trees/HSF/40 HSFs]). Around 2035, the final selection will be undertaken according to paternity analysis and experimental results of Experiments 3 and 4. Paternity analysis will be undertaken with molecular markers which generate many PCR fragments per primer, such as simple sequence repeat markers. Breeding values of the ST will be available for the establishment of our third-generation and it will then likely be beneficial to implement a PAM321 type strategy (e.g. ROSVALL et al., 2003; LSTIBÚREK et al., 2004) or a within-group PAM strategy. Given *L. kaempferi*'s low genetic variability compared to *L. decidua* (e.g. PARK and FOWLER, 1983; PÁQUES, 2001), it is likely that this species' genetic improvement will end after the second-generation. It will likely be more beneficial to use the same JL parents, whose characteristics will have been well defined (wood quality, disease resistance), to create several generations of H₁, while devoting greater efforts to EL improvement, if the SYN strategy is not retained. Finally, according to the results of Experiment 3, it would also be possible to continue the third-generation with the second-generation's preselected HL.

Production population

In order to quickly replace our second-generation HL varieties, around 2026, we will establish the third-generation of EL and JL production populations. The selection process will be

slightly different than that of the breeding population, while still ensuring that the trees are unrelated. For the two parental species, 16 unrelated HSFs will be selected on the basis of BLUP-estimated GHA values and the eight progeny tests' 10-year results. The best phenotype of each of these HSFs will be chosen by mass selection. In these EL and JL production populations, selection intensities will be high: 16 best phenotypes will be retained among 2048 candidate trees (128 trees/HSF/16 HSFs), *i.e.*, $i = 2.740$ or 0.8%. These 16 best phenotypes per parental species will make up production population A. In addition, the second best phenotype of these 16 top-ranked families will make up production population B. Population A parents will sometimes be replaced by their half-sib from population B, so as to maintain 12 to 16 parents per species in every seed production year.

Conclusions

Given that progeny testing of forest trees is expensive and time consuming, it is important to anticipate multiple objectives from the start. However, the study's dual purpose must not weaken its ability to address the relevant purpose. A good example is the integration of fundamental experiments within the generation of hybrid families needed for recommendations for vegetative propagation, presented here. Our empirical approach will increase the knowledge base involved in choosing a breeding strategy best adapted to optimizing the use of heterosis. Indeed, notwithstanding recent molecular genetic studies (e.g. AUGER *et al.*, 2005; SYED and CHEN, 2005), the mechanisms which underlie heterosis remain elusive. Like many complex phenomena, it does not seem that simple hypotheses will provide a solution. Indeed, results with rice (*Oryza sativa* L.; e.g. HUA *et al.*, 2003) and corn (*Zea mays* L.; e.g. SWANSON-WAGNER *et al.*, 2006) involve a number of genetic effects (dominance, overdominance and epistasis). One must hope that the integration of several complementary experiments, which include more fundamental objectives will provide benefits for our third-generation, as well as other interspecific tree breeding programmes. Finally, biological materials produced by these experiments can serve as the basis for future molecular genetic studies, to achieve a better understanding of molecular events resulting in heterosis in outcrossing trees, and also in furthering the study of this fascinating phenomenon.

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Annex I. – Description of genetic entries for the second breeding cycle of *Larix x marschlinii* in Québec, Can.

European larch (EL)			Japanese larch (JL)		
Source ¹⁾	No. of HSF ²⁾	No. of ST ³⁾	Source	No. of HSF	No. of ST
EL natural stand			JL natural stand		
Blizyn, Poland	1	4	Gunma prefecture, Japan	--	2
Kowary-Sniezka, Poland	1	4	Hokkaido, Japan	--	4
Secondary source			Secondary source		
Québec natural stand			Québec, natural stand		
Berthierville, Canada	2	8	Chatham, Canada	1	2
Drummondville, Canada	2	6	Seed orchard		
Seed orchard			Ganø, Lind., Denmark		
Clone 205, Germany	1	3	Kongenhus Flen., Denmark		
Two provenance tests			Morayshire, New., Scotland		
(Exp. 202-G-1 and 209-B-1)			MRNF-Q seed orchard		
Blizyn, Poland	3	4	Harrington, Canada		
Farum, Denmark	7	9	Lotbinière, Canada		
Grojec, Poland	4	6	Plantation		
Krakow, Poland	5	10	Ganø, Lind., Denmark		
Kroszowice, Poland	3	3	Ross-shire, Scotland		
Rundforbi, Denmark	2	2	Tokachi Hokk., Japan		
Schlitz, Germany	3	3	JL total		
Sckarzysko, Poland	2	6	12 and 80		
Wroclaw, Poland	5	9	13 prov.		
MRNF-Q⁴⁾ clonal bank					
Clone 158, Wisconsin, USA	1	3			
EL total	42	80			

¹⁾ Origin of the mother trees.

²⁾ HSF = half-sib family.

³⁾ ST = selected tree. All ST are of MRNF-Québec provenance and progeny tests and seed orchards (4 JL elite-trees; based on 5 years results from two progeny tests).

⁴⁾ MRNF-Q = ministère des Ressources naturelles et de la Faune du Québec.

Low Chloroplast DNA Diversity in Red Dogwood (*Cornus sanguinea* L.)

By H. LIESEBACH^{1),*} and B. GÖTZ²⁾

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Abstract

The red dogwood *Cornus sanguinea* L. is a deciduous shrub of the temperate and Mediterranean zones. It is often used in landscape gardening for miscellaneous purposes.

Chloroplast DNA markers, the so-called cpDNA haplotypes, are a very potential marker type to characterise the large scale variation pattern within the natural range of a species.

In this study, a total of 86 populations and 673 individuals were sampled all over Europe. Eight different haplotypes were recognised by combinations of several PCR-RFLP patterns. They are divided into 3 groups of related types. There is no association between these 3 groups and their geographic occurrence within the tested material.

One haplotype strongly dominates in the whole distribution area. It takes nearly 90 percent whereas the remaining seven haplotypes together reach to approximately 10 percent. Besides the low number of haplotypes, the total genetic variation $H_T = 0.15$ is much lower in *Cornus sanguinea* compared to other European tree and shrub species. Despite the low level of variation, several cases of introduced populations could be detected. Other haplotypes than the common type are found only in narrow areas. This result indicates that after the

¹⁾ Institute of Forest Genetics, Johann Heinrich von Thünen Institute (Federal Research Institute for Rural Areas, Forestry and Fisheries), Eberswalder Chaussee 3A, D-15377 Waldsiedersdorf, Germany.

²⁾ Forest Botanical Garden, University of Applied Sciences Eberswalde, Am Zainhammer 5, D-16225 Eberswalde, Germany.

*) Corresponding author: HEIKE LIESEBACH. Phone ++49(0)33433-157174, Fax ++49(0)33433-157199. E-Mail: heike.liesebach@vti.bund.de