Evaluation of the tree-improvement delivery system: factors affecting genetic potential

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Summary  Possible causes of the genetic erosion that occurs during the fragmented phases of the tree-improvement delivery system are reviewed. The impacts of intentional and unintentional directional selection during phenotypic selection, seed production (with its associated reproductive-phenology asynchrony, fecundity differential and varying propensity to inbreeding), seed processing and storage, and seedling production are evaluated. In general, genetic diversity and heterozygosity parameters of seed orchards are higher or similar to those observed in their natural-population counterparts. However, parental contribution to the resultant seed orchard seed crops is consistently asymmetrical, and this is a major cause of genetic erosion. In most cases, less than 20% of an orchard’s clones contribute 80% of the cone crop, thus reducing the effective population size. Because seed germination of coniferous tree species is under strong maternal genetic control, the combined effects of differences in reproductive output and germination, as well as of management practices (e.g., simulated long-term storage of seed showed that loss of viability during storage is genotype specific), cause unintentional directional selection during seedling production. This review confirms the need for genetic monitoring of each phase of the tree-improvement delivery system, so that practical solutions can be developed to alleviate genetic erosion.

Keywords: closed populations, genetic erosion, genetic variation, inbreeding, parental contribution, phenotypic selection, reproductive output, reproductive phenology, seed production, seed storage, seedling production.

Introduction

The reduction of genetic variation in populations with a relatively limited number of breeding individuals (closed populations) is a well-established fact of modern population genetics theory. Thus, in closed populations, the number of breeding individuals and their fitness play a pivotal role in long-term survival and evolutionary adaptation to changing environments. In domesticated species, the importance of maintaining and conserving genetic variation increases with the expected progressive reduction of breeding individuals. Loss of genetic diversity through domestication is well documented in agricultural crops (see Francis 1981); however, it is not clear to what extent domestication (Figure 1) affects the genetic diversity of forest tree populations. The domestication of wild species has the potential to affect the rate of genetic change more than that expected under natural evolutionary processes. Because coniferous trees have a long life span, the maintenance of high levels of genetic diversity in breeding and production populations is essential for their viability, survival and evolution (Hamrick and Godt 1989).

I have evaluated the three major steps of the domestication process in forest trees (i.e., tree-improvement delivery system), namely phenotypic selection, seed production and seedling production, to determine how they affect genetic diversity.

Phenotypic selection

The relationship between population size and genetic variability is critical to the domestication of wild, long-lived species. A reduction or a crash in population size is usually associated with a reduction in genetic variability (Frankel and Soulé 1983).
In agricultural crops, it is well known that losses in genetic diversity are associated with genetic-improvement programs; however, no assessment of potential losses in forest tree species has been made. If isozyme genotypes (biochemical finger-prints) are not associated with the phenotypic or quantitative attributes of individuals, then they present a unique set of neutral, random, gene markers for monitoring genetic variation through the process of “parent-tree selection.”

I used allozyme analysis to compare the heterozygosity of first-generation seed orchards of three conifers with their corresponding natural populations. The species compared were western red cedar (Thuja plicata J. Donn ex D. Don), Sitka spruce (Picea stichensis (Bong.) Carr.) and Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) (Table 1). A comparison of the heterozygosity parameters (the number of alleles per locus, percentage of polymorphic loci and mean heterozygosity (Hartel 1980)) between the seed orchards and their corresponding natural populations’ pooled data indicated that the amount of genetic variation present in natural populations was retained or increased during the phenotypic-selection step (Table 1). This is because, although the sampling of natural populations is extensive, it is usually limited to a relatively small area, such that trees sampled for breeding may include other alleles not originally sampled. This finding is also concordant with the observed clustering of similar genotypes within natural forest tree populations. The reason why the frequently observed reduction of genetic diversity in agricultural crop plants was not observed in the three conifers studied is not known, but it may be related to the fact that conifers are still in the first stage of domestication and have a high genetic variability (Hamrick and Godt 1989).

### Seed production

Seed orchards represent the link between breeding programs (i.e., the product of phenotypic selection) and reforestation activities (i.e., the product of the breeding–testing–selection cycle) through the consistent delivery of genetically improved seed. Because seed orchards normally consist of a restricted number of genotypes, there is potential for loss in genetic variability along this link. Attainment of genetic gain through selection and breeding is dependent on maintaining the frequency of desirable genes in the seed-orchard crops at the level present in the selected parent population comprising the seed orchard. This goal requires the attainment of the random-mating assumption of the Hardy-Weinberg theorem. Panmictic equilibrium is achieved only when the seed orchard’s clones are in reproductive synchrony and contribute similarly to the gene pool (i.e., equal reproductive output). There are various biological factors affecting panmictic equilibrium in seed orchards including the pattern of reproductive phenology, reproductive output and inbreeding.

### Reproductive phenology

Synchrony of reproductive phenology among a seed orchard’s clones is a prerequisite to a successful seed-orchard crop. Failure to achieve synchrony of reproductive phenology affects the panmictic equilibrium of the seed orchard (Eriksson et al. 1973). It is well known that both time and temperature, summarized as heat sum, have a significant effect on reproductive phenology (Worrall 1983). Because seed orchards consist of several genotypes that are usually adapted to different environmental conditions, with various heat-sum requirements for bud burst, time differences in reproductive bud phenology will occur among the orchard clones (Figure 2). When differences in the timing of reproductive phenology exist, the orchard clones form several temporally isolated breeding subpopulations thereby increasing the rate of inbreeding, reducing panmixia and increasing the likelihood that the early and late flowering classes will be exposed to nonorchard pollen (El-Kassaby and Ritland 1986a). Synchrony of reproductive phenology in several seed orchard species was investigated and, with the exception of western red cedar (El-Kassaby 1992), all displayed an extended pollination season and subdivision of the seed orchard population into several temporally isolated breeding subpopulations (El-Kassaby et al. 1984, Fashler and El-Kassaby 1987, El-Kassaby and Reynolds 1990). This sub-

### Table 1. Comparison of heterozygosity parameters in seed orchards and natural populations.

<table>
<thead>
<tr>
<th>Species</th>
<th>Percent polymorphic loci</th>
<th>No. of alleles per locus</th>
<th>Mean expected heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western red cedar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed orchard (n = 1)</td>
<td>11.1</td>
<td>1.2</td>
<td>0.058</td>
</tr>
<tr>
<td>Natural populations (n = 2)</td>
<td>11.1</td>
<td>1.1</td>
<td>0.055</td>
</tr>
<tr>
<td>Sitka spruce</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed orchard (n = 1)</td>
<td>100.0</td>
<td>2.8</td>
<td>0.229</td>
</tr>
<tr>
<td>Natural populations (n = 10)</td>
<td>66.9</td>
<td>1.8</td>
<td>0.183</td>
</tr>
<tr>
<td>Douglas-fir</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed orchard (n = 12)</td>
<td>62.5</td>
<td>2.3</td>
<td>0.172</td>
</tr>
<tr>
<td>Natural populations (n = 49)</td>
<td>32.6</td>
<td>2.1</td>
<td>0.171</td>
</tr>
</tbody>
</table>

1 El-Kassaby et al. 1993a.
2 Chaisurisri and El-Kassaby 1994.
3 El-Kassaby and Ritland 1995.
division also affected seed yield. For example, El-Kassaby and Reynolds (1990) observed large reductions in seed set in a Sitka spruce seed orchard where maximum pollen release occurred 10 days after the commencement of seed cone receptivity. Based on the length of the pollination season, the presence of a disproportionate number of pollen donors throughout the pollination season (El-Kassaby et al. 1984), and the short duration of seed cone receptivity and pollen-shedding in relation to the reproductive-phenology profile, El-Kassaby et al. (1984) concluded that most orchard populations are in panmictic disequilibrium. Under this condition, the seed orchard's population is a continuum of small breeding subpopulations throughout the pollination season. This reduces maximum exchange of genes among the different genotypes and leads to a decrease in the genetic diversity of the resultant seed crop.

Differences in the timing of reproductive phenology can affect the panmictic equilibrium of seed orchards in several ways. Under panmixia, the expected selfing rate is equal to \( \frac{n}{n^2} \), where \( n \) is the number of clones in the orchards. Therefore, in a hypothetical orchard that consists of 30 clones, the expected selfing rate is 3.33%. If this orchard is divided into three nonoverlapping breeding subpopulations with equal (1/1/1) or unequal proportions (1/4/1) of clones, the expected selfing rates will be 10 and 6.6%, respectively. This hypothetical orchard will produce three- and twofold differences in selfing over that expected when a panmictic equilibrium exists, thus increasing the chance of reductions in genetic variation.

Reproductive output

The maintenance of similar allelic frequencies in a seed orchard and its seed crop is dependent on the presence of balanced gametic production among the orchard's clones and absence of effective selection involving the alleles studied. The parental balance of seed orchards is often based solely on seed-parent contributions. The so-called “20/80” rule (i.e., 20% of the clones produce 80% of the cone crop) was coined by the North Carolina State Tree Improvement Cooperative to describe the parental balance in seed orchards (Anonymous 1976). Parental-balance values based on seed-parent contributions have become an accepted method to evaluate and even to provide genetic rating of seed-orchard crops. However, parental-balance estimates of several studied seed orchards have revealed that the parental contribution to cone and seed crops varies substantially among a seed orchard’s clones (Griffin 1982, O’Reilly et al. 1982, Byram et al. 1986, El-Kassaby et al. 1989, El-Kassaby and Reynolds 1990, Reynolds and El-Kassaby 1990, Chaisurisri and El-Kassaby 1993) (Figure 3).

In most cases, a small proportion of clones contribute the majority of cone/seed crops, thus reducing the expected genetic variation and producing crops with unpredictable allelic frequencies. Chaisurisri and El-Kassaby (1993) demonstrated that the concept of effective female population number (Crow and Kimura 1970) can be used to show deviation from the ideal case of equal contribution. In their study, cone counts were used to determine the effective female population size. The proportion of female effective and actual numbers \( \left( \frac{n_e}{n} \right) \) was also estimated, producing a value of 0.45 which is much smaller than that expected under the equal-contribution assumption. Male reproductive success was also investigated for

![Figure 2. Pattern of reproductive phenology in a western hemlock (Tsuga heterophylla) seed orchard.](image)

![Figure 3. Cumulative seed-cone production curves for a good cone year (1983) and a poor cone year (1981) (El-Kassaby et al. 1989).](image)
Douglas-fir and loblolly pine (*Pinus taeda* L.), and distorted contributions to the gametic pollen pool were found, further reducing the genetic diversity (Roberds et al. 1991, El-Kassaby and Ritland 1992, Nakamura and Wheeler 1992).

**Inbreeding**

Although estimates of natural outcrossing in conifers are high (*t* > 0.9, Adams and Birkes 1990), even a low level of inbreeding in seed orchards is important because most forestry programs rely on noncompetitive plantings in both nursery-production and plantation-establishment phases.

In general, estimates of outcrossing rates for seed orchards are higher than those reported for natural stands of the same species, suggesting that population structure (i.e., the physical arrangement of related individuals within a population) affects the rate of outcrossing. If the arrangement of an orchard population can increase the rate of outcrossing, then additional manipulation of pollen dynamics could further reduce inbreeding, and consequently increase heterozygosity in the seed crop. The use of an overhead-cooling treatment in coastal seed orchards has been successful as a method for preventing pollen contamination (El-Kassaby and Ritland 1986b) and has produced additional benefits, including a large reduction in reproductive-phenology differences (Fashler and El-Kassaby 1987). The duration of flowering and pollination in cooled seed orchards is shorter and outcrossing rates are higher than in uncooled seed orchards (El-Kassaby and Davidson 1990, 1991).

In summary, the extent of genetic variability in seed orchard crops is greatly affected by the interplay among reproductive phenology, reproductive output and inbreeding. The maintenance of high levels of genetic variability during seedling production is dependent on a detailed understanding of seed biology.

**Seed biology**

Seed-orchard seed crops represent the vehicle by which the genetic gain of tree-improvement programs is transmitted to new generations. In most cases, seed-orchard crops are collected, extracted and stored in bulked seedlots. Bulked seedlots are usually composed of undetermined proportions of seed from each of several seed parents.Treating a collection of seed produced from different genotypes uniformly during extraction, germination and storage assumes that all seeds will respond to the treatments in a uniform fashion. However, recent studies on the genetics of conifer seed size, dormancy and germination have demonstrated that genotypic differences represent a major and important component of the total variation observed for these attributes (Chaisurisri et al. 1992, El-Kassaby et al. 1992, 1993b), indicating that a knowledge of the genetic differences in seed traits would be beneficial in the maintenance of the genetic diversity of seed crops. Broad-sense heritability estimates of germination parameters for western red cedar, Sitka spruce and Douglas-fir are presented in Table 2. Because all germination parameters of these species produced high heritability estimates, which are indicative of the degree and extent of genetic control over these attributes, it is expected that any uniform dormancy-breaking treatment applied to bulked seedlots will produce different responses because of the different genetic backgrounds of the seedlots (Figure 4). Furthermore, because germination rate is under genetic control, the different genotypes will germinate at different rates, resulting in a large variation during emergence (Figure 5).

Seed viability is highest at the time of physiological maturity and declines with age. The time courses for seed deterioration range from days to years. In agricultural crops, it has been demonstrated that genotypes differ in the degree of seed viability loss during storage (Delouche and Baskin 1973). If conifer seed crops are collected, extracted and stored on a bulk basis, then seed-viability losses by a specific genotype cannot be traced, and the reduction in total genetic diversity passes undetected (El-Kassaby 1992). The effects of viability loss in Sitka spruce, Douglas-fir, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) and lodgepole pine (*Pinus contorta* Dougl. ex Bong.) seed were assessed by a simulated-aging technique known as accelerated aging (Delouche and Baskin 1973). In this test, seeds are exposed to a range of temperatures and humidities for varying times. In all the species investigated, germination rate declined steadily with time of exposure; however, the rate of decline varied among the genotypes (El-Kassaby 1992).

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**Table 2. Estimates of broad-sense heritabilities (h²) for germination capacity (GC), peak value (PV) and germination value (GV) for western red cedar, Sitka spruce and Douglas-fir.**

<table>
<thead>
<tr>
<th>Species</th>
<th>GC</th>
<th>PV</th>
<th>GV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western red cedar</td>
<td>0.79</td>
<td>0.78</td>
<td>0.80</td>
</tr>
<tr>
<td>Sitka spruce</td>
<td>0.42</td>
<td>0.78</td>
<td>0.74</td>
</tr>
<tr>
<td>Douglas-fir</td>
<td>0.92</td>
<td>0.91</td>
<td>0.93</td>
</tr>
</tbody>
</table>


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**Figure 4. Germination curves for 19 stratified Douglas-fir families (El-Kassaby et al. 1992).**
Chaisurisri et al. 1993) (Figure 6). These differences in the rate of viability loss indicate that the genetic makeup of bulked seedlots changes during storage. If seedlots were harvested, extracted and stored on a clonal basis, then reductions in genetic variability caused by viability losses could be identified by periodic tests.

Seedling production

Maximizing diversity in orchard crops is at odds with the uniformity required for large-scale commercial production of seedlings. Maximizing the number of plantable seedlings per unit area is paramount if container nurseries are to reach their maximum economic potential. If the seed sources used are bulked seedlots, then biological factors such as differences in parental contribution and germination parameters may affect the genetic diversity of the crop. In a bulked seedlot, seed viability is the weighted average of the viabilities of the individual clones contributing to the seedlot. In addition, if clones vary in their rates of germination, the practice of thinning multiple germinants per cavity (i.e., leaving the largest germinating clone) will result in unintentional selection pressure in favor of the faster germinating clones (El-Kassaby and Thomson 1990).

Conclusions

Systematic monitoring of genetic variation through the fragmented processes of some conifer tree-improvement delivery systems has identified several sources of genetic erosion resulting in a reduction of the genetic potential of these programs. The causes of genetic erosion were identified and the importance of understanding the underlying genetic control and the biological peculiarities of these processes were emphasized. The fragmented, horizontal organization of the tree-improvement delivery system requires evaluation and modification so that genetic diversity is not affected during the process.

Acknowledgments

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References


