Genetic Conservation in Applied Tree Breeding Programs

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Abstract. This paper reviews how population size and structure impacts the maintenance of genetic variation in breeding and gene resource populations. We discuss appropriate population sizes for low frequency alleles and point out some examples of low frequency alleles in the literature. Development of appropriate breeding populations and gene resource populations are discussed.

Introduction

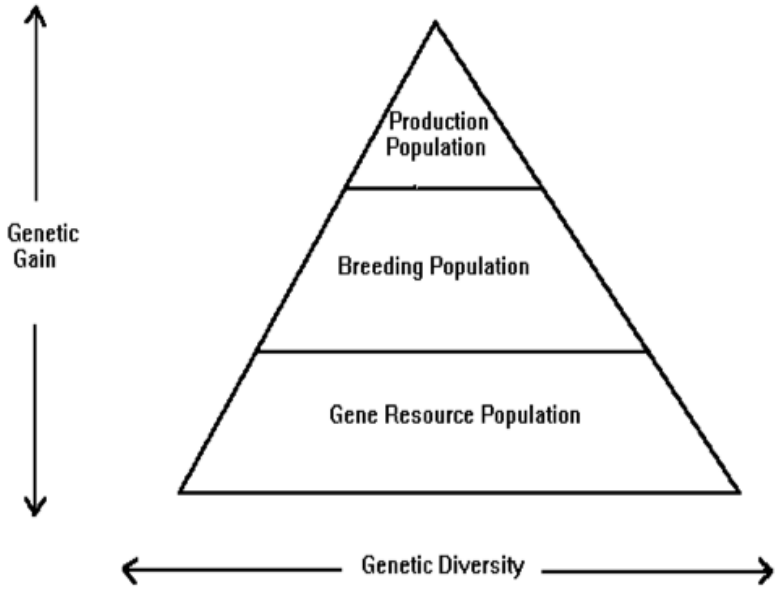
The primary objective of a breeding program is to increase the frequency of desirable alleles found in the breeding population. While breeders know the traits they wish to improve, they do not know which alleles (genes) favorably impact the traits or their distribution in the native population. Breeding programs must maintain sufficient genetic variation to allow for continued genetic gains over multiple generations. Complicating matters is the fact that traits of interest change over time in response to new pests or changes in markets. Population sizes needed to maintain gains in polygenic traits of current interest are much smaller than population sizes needed to find potentially rare traits that may be desired in the future. This paper considers the impact of breeding population size and structure on the maintenance of genetic variation and on continued genetic gain.

The breeder needs to consider both short- and long-term objectives when structuring a breeding program. Short-term objectives usually include obtaining substantial gains in current traits of interest in the first few generations of breeding while maintaining well-adapted trees. Long-term objectives include the maintenance of low frequency alleles and control of inbreeding. A major conflict arises between short- and long-term objectives. Selection intensity must be high to obtain substantial genetic gains, yet maintaining rare alleles requires keeping a large breeding population in subsequent generations. However, there are ways to structure the breeding population and make selections to reduce this conflict.

Fortunately, the breeding population is only one aspect of gene resource management. Rowland Burdon developed a pyramid that conceptualizes the role of the breeding population in gene resource management (Figure 1). The horizontal axis represents genetic diversity and the vertical axis represents genetic gain. The gene resource population represents all of the available genetic variation that could contribute to the breeding population. This includes native stands, provenance trials, seed orchard parents, progeny in progeny tests, and operational plantations. The next level is the breeding population, which must have sufficient genetic variation to maintain genetic gain for multiple generations. It tends to be more improved than the gene resource population. At the top is the production population, consisting of seed

orchard candidates or clones used for operational deployment. These selections are the best selections from the breeding population and provide diversity and genetic gain to operational plantations.

Figure 1. Conceptualization of gain versus genetic variation for a gene resource management program.



While it would be nice to maintain all genetic diversity in the breeding population to account for unforeseen contingencies, this is not possible. Thousands of parents are required to maintain low frequency alleles for many generations (Millar and Libby 1991, Lynch 1995, Lande 1995, Yanchuk 2001), and breeding populations of this size are not financially or practically feasible. Breeders can make intelligent, or at least informed decisions by understanding which alleles are being affected by selection and by understanding the genetic variation within a species.

Population size and the conservation of genetic variation (alleles)

Unless population size is infinite, alleles are lost; it happens in nature all the time. The probability of a neutral allele being maintained in a population is primarily a function of the initial allele frequency and the effective population size. Effective population size (Ne) is an estimate of the number of individuals that would give rise to the sampling variance or the rate of inbreeding for the appropriate number of random mating parents with equal contribution of every parent (Falconer and Mackay 1996, pg. 65). Ne is determined by the number of selections and their relatedness.

When deciding on a population size for a breeding or gene resource population, it is important to consider the probability of allele loss for varying allele frequencies and the associated risks. The probability of allele loss can be calculated using

appropriate formula. Gregorius (1980) examined this probability for a population in Hardy-Weinberg equilibrium. Namkoong (1988) presented tables examining the minimum number of genotypes required to allow for the loss of only one allele when considering differing numbers of loci and alleles. Kang (1979a) modified a formula from Kimura and Ohta (1969) to determine the population size needed to maintain a neutral allele with specific initial allele frequencies. A general formulae (found in Frankel *et al*. 1995, p 36) for the sample size needed to be 95% certain of obtaining one copy of an allele with population frequency of p with an inbreeding coefficient of F is $3/\{(F-2)\log_e(1-p)\}$. Thus, to maintain at least one copy of an allele with a frequency of 0.05 for multiple generations, a maximum population size of 117 appears to be necessary (Table 1, Kang=79, Gregorius=117, Namkoong=117, Frankel *et al*.=59). For alleles at frequencies of ≥0.20, the number is reduced to 31 (Table 1, Kang=31, Gregorius=21, Frankel *et al*.=14).

Table 1. Population size recommended for maintaining neutral alleles in a population.

Allele frequency	Kang (1979) [1]	Gregorius (1980)[2]	Namkoong (1981)[3]	Frankel *et al.* (1995)[4]
0.5	18	6		5
0.2	31	21		14
0.1	49	51		29
0.05	79	117	117	59
0.01	269	754	597	299

1 - Population size necessary to maintain neutral alleles for 50 generations
2 - Minimum sample size required to ensure all alleles at a locus are detected with 95% probability
3 - Minimum number of genotypes required for a average loss of one allele at any of 100 loci with 4 rare alleles per locus
4 - Sample size needed to be 95% certain of obtaining one copy of a gene with for a population with an inbreeding coefficient F=1.

Practically, these numbers must be increased for several reasons. They assume neutral alleles, but specific alleles may be linked to loci being selected against. Also, "population size" refers to the effective population size (Ne), which is always less than the census number. To account for this, Namkoong and Roberds (1982) suggest doubling the calculated effective population sizes. Additionally, Yanchuk (2001) points out that one copy of an allele would not be useful to most breeding programs. Instead, breeding programs would want closer to 20 usable copies of a gene so that inbreeding can be controlled; this requires that that the allele be evident in 20 phenotypes. Very large population sizes are needed to find 20 phenotypes for recessive alleles. A population size of 2,784 is needed if the recessive allele is at a frequency of 0.1, while 278,788 is needed if the frequency is 0.01 (Yanchuk 2001).

Maintaining low frequency alleles is important to the very long-term maintenance of populations and the genetic conservation literature has many

suggestions as to how many individuals (Ne) are needed to maintain a population and its inherent genetic variation for hundreds of generations. Franklin (1980) and Soulé (1980) suggested that 500 individuals (Ne) provide the needed genetic variation. Lynch (1995) suggested an Ne of 1,000, whereas Lande (1995) calculated an Ne of 5,000. Millar and Libby (1991) suggested that effective population sizes (Ne) of 2,230 to 9,110 are needed to maintain heterozygosity levels (H_e) for ponderosa pine and Douglas-fir.

It is unrealistic to think we will be able to preserve alleles with extremely low frequencies in the breeding population. These alleles are probably at low frequency because they are not presently beneficial; although, in the future they may be of use. Low frequency alleles are much better conserved in the gene resource population.

The breeding population: maintaining alleles that contribute to gain in the short term

The most efficient way to achieve gain in early generations of a tree breeding program is to start with the most appropriate population or land race. Most species have substantial among-provenance variation, so that beginning with the best provenance or land race is important. This requires that information from provenance trials be available. Eldridge et al. (1993) provide an excellent discussion of the value of provenances and land races.

The appropriate number of initial selections and families needed to obtain short-term gain has been addressed recently by Lindgren et al. (1997). Because many traits have low to moderate narrow sense heritabilities, we depend upon among-family selection to provide a substantial part of the gain. One needs to start with large numbers of initial selections and families to allow for intense family selection to ensure early gain. Lindgren et al. (1997) suggest that 200 unrelated parents is a reasonable number to achieve gain when considering the costs and benefits of breeding.

Selection has the greatest impact on allele frequencies in the intermediate range (0.25 to 0.75) (Namkoong 1979, Falconer and Mackay 1996). These alleles are of primary importance for genetic gain in the first 5 to 10 generations of breeding, assuming that selection is for polygenic traits like growth. Based on the discussion above, 30 to 50 individuals in a breeding program is sufficient to ensure that the genes most influenced by selection will be maintained in the breeding population. In later generations, favorable alleles that were initially at low frequencies will be in the intermediate range and contribute the most to genetic gains. The genes that were initially at intermediate frequencies will be closer to fixation and not as important to achieving gains.

Baker and Curnow (1969) demonstrated that an initial Ne of 16 enables nearly as much gain as an Ne of 256 in the first five generations of breeding; very few favorable alleles are lost to random drift (random sampling) (Table 2). An Ne of 32 is almost as good as an Ne of 256 for 10 generations. Namkoong et al. (1989) also suggested that population sizes as small as 20 are adequate when dealing with limited generations. Kang (1979a) showed that an Ne of 17 would probably suffice to fix genes at a frequency of 0.25 and above.

Table 2. Expected progress (gain) from selection after 1, 5, 10, and infinite (∞) generations of selection for different values of Ne in a model population (Baker and Curnow 1969).

Ne	Generation			
	1	5	10	∞
4	3.3	12.4	19.5	28.4
16	3.3	16.0	31.4	114.5
32	3.3	16.8	34.6	177.5
64	3.3	17.2	36.4	220.9
256	3.3	17.5	37.8	240.0
∞	3.3	17.6	38.0	240.0

It would appear that if a breeder was only concerned about gain for 10 generations that an Ne of 30 to 50 would suffice for a single trait, provided breeding objectives did not change. However, most programs breed for multiple traits and traits of interest do change over time. Furthermore, the variance of the response must be considered. Smaller populations may have high expected gains, but the variability of those predicted gains can be high (reviewed by White 1992).

Gene resource populations: maintaining variation so that new traits can be incorporated in the future

The above discussion assumes that the traits of interest to tree breeders will remain constant. We do not foresee breeding programs losing interest in improving growth rate, but history shows that new traits are often desired. Examples of traits added to breeding programs include wood density, tree form, pulping characteristics, and disease and insect resistance. Most new traits will probably be polygenic and have genetic variation in the breeding population. The typical impact of selecting for additional traits is that gain in current traits is reduced. However, major difficulties arise when a new trait is controlled by alleles at very low frequencies or when the alleles can be found only in a few localized populations.

Examples of low frequency alleles

One example of a low frequency desirable allele is the MGR gene for blister rust resistance in *Pinus lambertiana* (Kinloch 1992). Over the range of the species, the frequency of this allele is 0.022; however, within individual seed zones the frequency varies between 0 and 0.087. Using the population wide frequency of 0.022, the population size needed to maintain and observe at least 20 phenotypes of this dominant allele is approximately 600 (Yanchuk 2001). Had the allele been recessive, over 55,755 individuals would be needed to observe 20 phenotypes with 95% probability.

Fortunately, at least in crop breeding, disease resistance genes are usually dominant, although they are recessive around 10% of the time (Burdon 1987, in Burdon 2001).

Another example of a low frequency allele that is valuable to tree breeders is the allele that alters lignin properties in *Pinus taeda* (Ralph et al. 1997). This allele has only been demonstrated in one first-generation parent.

Examples of traits found in localized populations

Finding desirable genes or genotypes is further complicated when they occur only in isolated populations. Examples of pest resistance found only in specific populations include the MGR gene for blister rust resistance in *Pinus monticola* (Kinloch et al. 1999) and white pine weevil resistance in *Picea sitchensis* (Ying 1991, 1997). In the case of *Picea sitchensis*, resistance occurs predominantly in two British Columbia populations. These populations would not usually be considered for breeding in Oregon because growth rates of the local selections are much faster than those from British Columbia. Because very little resistance is evident in Oregon, resistant selections from British Columbia will be incorporated into Oregon breeding populations. An example of a wood property trait is wood density in the Guadalupe Island population of *Pinus radiata*. This island population has considerably higher core wood density than the New Zealand land race or the three mainland populations (Burdon and Low 1992, Low and Smith 1997).

Implications for breeding programs

The above discussions point out that, although 200 initial selections in a breeding program may maintain gains when breeding objectives do not change, many more individuals may be necessary to maintain genetic diversity for novel traits, particularly when those traits are rare. Most breeding populations have between 200 and 400 selections, although some have as many as 1,000 selections (Table 3, updated from White 1992). Most programs cannot afford to double their population sizes in order to maintain alleles like the MGR gene in *Pinus lambertiana*. Likewise, keeping poorly adapted provenances in a breeding program is a high price to pay to maintain genetic diversity. It becomes obvious that low frequency alleles must be maintained in gene resource populations. Levels of gain for current traits of interest in a gene resource population will be lower than in a breeding population. Therefore, one should expect a reduction in gain when genotypes from the gene resource population are incorporated into the breeding and production populations.

Table 3. Approximate census number (N) for breeding populations of some advanced-generation tree improvement programs. N is on a "per breeding unit" basis for programs with multiple breeding units (updated from White 1992).

Species	Program	N	Citation

Eucalyptus globules	CELBI - Portugal APM - Australia	300 300	Cotterill *et al*. 1989 Cameron *et al.* 1989
Eucalyptus grandis	ARACRUZ - Brazil	400	Campinhos and Ikemori 1989
Eucalyptus nitens	APM - Australia New Zealand	300 270	Cameron *et al*. 1989 Gea et al. 1997
Eucalyptus regnans	APM - Australia New Zealand	300 300	Cameron *et al*. 1989 Cannon and Shelbourne 1991
Eucalyptus urophylla	ARACRUZ - Brazil	400	Campinhos and Ikemori 1989
Picea glauca	Nova Scotia – CAN	450	Fowler 1986
Picea mariana	New Brunswick - CAN	400	Fowler 1987
Picea abies	Sweden	> 1000	Rosvall et al. 1998
Pinus banksiana	Lake States – USA Manitoba – CAN	400 116	Kang 1979 Klein 1995
Pinus caribaea	QFS – Australia	200-300	Kanowski and Nikles 1989
Pinus elliottii	CFGRP – USA WGFTIP – USA	900 800	Hodge *et al*. 1989 Lowe and van Buijitenen 1986
Pinus radiata	STBA – Australia FRI – New Zealand	300 550	White et al. 1999 Jayawickrama and Carson 2000

Pinus taeda	NCSU – USA WGGTIP – USA	160 800	McKeand and Bridgwater 1998 Lowe and van Buijitenen 1986
Pseudotsuga menziesii	BC – CAN NWTIC - USA	450 0 - 404	Woods 1993 Annon. 2001
Tsuga heterophylla	HEMTIC CAN-USA	150	King and Cartwright 1995

A risk analysis should be carried out by an organization to weigh the costs and potential benefits of enlarging a breeding program to maintain genetic diversity. For example, in a simple simulation examining a number of different breeding options, Johnson (1998) showed that strategies with the highest Ne were not always optimal for achieving gain after needing a low frequency allele.

Maintenance of genetic diversity in gene resource populations

There are several types of gene resource populations, traditionally catagorized as either *in situ* or *ex situ*. *In situ* techniques involve conserving genetic resources in native habitats, while *ex situ* techniques involve storing genetic resources in special collections such as seed banks, progeny or provenance tests, and seed orchards. Both *in situ* and *ex situ* management are important in maintaining genetic diversity for a breeding program. They vary in effectiveness depending on objective, species, origin, management intensity, population size, etc. One important measure of effectiveness is whether an organization has control of the particular gene resource population. Because these are long-term populations, one needs some control over these populations to ensure they will be available in the future.

In situ reserves tend to be the less costly option for maintaining genetic diversity, but the difficulty with this approach is that most organizations have little control over the *in situ* reserves throughout a species' range. However, it is usually possible to monitor the *in situ* reserves in order to decide when *ex situ* measures are needed. Such efforts are underway in the Pacific Northwest of North America. In British Columbia, the BC Ministry of Forests has inventoried *in situ* populations in an effort to find populations that may be at risk (Lester and Yanchuk 1996). In Oregon and Washington, numerous organizations have come together to do a "gap" analysis of eight important conifer species (St. Clair and Lipow 2000). The cooperative approach minimizes the costs to any one organization.

Ex situ genetic conservation programs may also be carried out by multiple organizations working cooperatively. Examples include the provenance studies organized by IUFRO in the past and the current efforts of the Central America and Mexico Coniferous Resources Cooperative (CAMCORE). CAMCORE is a cooperative organization that is working to establish *ex situ* gene resource populations of tropical species, many of which are threatened. Presently, 24 organizations are

members of the cooperative. *Ex situ* populations have been established for 22 conifer and 13 hardwood species (Dvorak et al. 1996, http://www2.ncsu.edu/camcore/index.htm).

Provenance studies are important to breeding programs, but most provenance studies are not suitable as "stand alone" gene resource populations. If a desired allele is identified in only one provenance, there will probably be fewer than the 20 unrelated copies recommended by Yanchuk (2001), because any one provenance is usually represented by a limited number of parents. Although provenance trials can only provide limited genetic variation to a breeding program, they are important because they can show the geographic distribution of a trait. For example, it was in provenance trials where the weevil resistance populations of *Picea sitchensis* were first identified (Ying 1991).

Ex situ conservation is extremely important when an organization is breeding exotics and the species is in jeopardy within its native range. One example is the collections and plantings made by Australia and New Zealand of *Pinus radiata* (Libby et al. 1966, Eldridge 1979, see also Matheson et al. 1999). The natural distribution of *Pinus radiata* is limited to five relatively small populations. Collections of these populations are planted in large blocks in both countries with management plans in place.

Many breeding programs are using their first generation selections as gene resource populations. For example, the Western Gulf Forest Tree Improvement Program has grafted all of its first generation *Pinus taeda* selections into scion banks (Byram et al. 1999). A similar conservation program was established with *Bombacopis quinta* by Monterrey Forsestal (Vallejo 1999). The Northwest Tree Improvement Cooperative (NWTIC) in Oregon and Washington USA is using their first generation progeny tests as gene resources populations (Lipow et al. in mans.). Because progeny tests will not survive indefinitely, methods are being discussed to regenerate stands to maintain these populations in the long term as multiple populations, as suggested for *Pinus taeda* by Namkoong (1997).

In Europe, gene conservation programs have been proposed that use both *in situ* and *ex situ* populations in different multiple populations (Eriksson 2001). Use of multiple populations conserves genetic variation better than a single population of the same size as the sum of the multiple populations (Namkoong 1984).

Suggestions in developing ex situ gene conservation populations

Before constructing *ex situ* gene resource populations, it is informative to evaluate the status of *in situ* reserves; are they in danger? Yanchuk and Lester (1996) considered a "population" adequately protected if it was represented by more that 5,000 m^3 of wood. They assumed 0.5 m^3 per tree, resulting in at least 5,000 trees. The definition of a "population" used by Yanchuk and Lester (1996) was a biogeoclimatic zone, which are used as seed zones in British Columbia. An organization should also be aware of *ex situ* populations that may be available, in cases like *Pinus radiata*, the species is probably best conserved in *ex situ* populations as long as they are appropriately regenerated.

If an organization decides that a valuable population is in danger, then establishment of *ex situ* gene resource populations may be warranted. Burdon (1986,

1995) discusses issues involved in developing *ex situ* populations. We would suggest that a minimum of 50 unrelated selections per population be used to establish a gene resource population. This would ensure the capture of genes with frequencies of 0.1 and greater. These populations should be established in stands of 1,500 stems or more. Multiple stands should be established to spread the risk of losing a population during a natural disaster or mistaken harvest. They should also be established in such a way that they grow for many years.
The longer the life of the stand, the longer it will be before one needs to collect seed and reestablish new stands.

By planting and identifying family rows one can control the female genetic component of seed collections in the future. This may lead to some level of inbreeding from sib pollination, but it would be impractical to map single-tree plots in these populations so that one could identify maternal parents in the future. A small amount of inbreeding would not necessarily be bad because the objective of these populations is to conserve genes and gene complexes, not necessarily to "improve" the population. Any inbreeding depression can be removed by outcrossing in one generation. If regeneration plans depend on wind pollination rather than control pollination, larger stands should be considered to ensure that the pollen component in the next generation is from the appropriate provenance or population.

Maintaining genetic variation in breeding populations

Programs can also manage their breeding populations to better hold on to genetic variation while still obtaining genetic gain. Making wise decisions in early generations is crucial in maintaining the genetic variation needed later.

Breeding population structure

One way to maintain genetic variation in the breeding population is to structure it in subpopulations. There have been two basic methods proposed to structure breeding populations; either selections are stratified by their genetic value or they are stratified by their selection goals or geographic origins. These two methods are reviewed by Eriksson et al. (1993), Williams et al. (1995) and Williams and Hamrick (1996).

One method of structuring a population is to stratify the breeding population based on genetic merit. The very best selections are placed into the elite population and the remainder of the breeding population is placed in the "main population". The idea is to concentrate more effort on the elite population, where maximum gain is expected, and less on the main population. Ways to emphasize the elite population include making more crosses with parents, testing families on more sites and turning generations faster. The main population serves as both a breeding population and a gene resource population. This system was initially used in maize (Kannenberg 1981) and sheep breeding (James 1977), and later incorporated into forest trees by Cotterill et al. (1989). Such programs are referred to as nucleus breeding programs (James 1977 and Cotterill et al. 1989) or hierarchical open ended (HOPE) programs (Kannenber 1981). Lindgren and Matheson (1989) proposed a strategy using a similar concept for seed orchards. They suggested that clones be used in proportion to their breeding value, with better clones having more ramets in the seed orchard. This idea was

described for breeding programs by Kang (1989) and Kang and Namkoong (1988) where the better clones (parents) would be used to make more crosses than the poorer clones.

The use of "multiple populations" has been proposed as a way of better maintaining genetic variation in a breeding program. The multiple population breeding strategy refers to having many subpopulations of relatively small size (20-50) designed to maintain genetic diversity in the breeding population. The concept was introduced to forestry by Namkoong (1976, 1984) to account for uncertainty in the future value of selected traits. The idea is that each multiple population is selected for different traits (or different weightings), thus providing more options (genetic variation) in the future. Multiple populations, each selecting for different traits, will conserve genetic diversity better than one single breeding population (Namkoong et al. 1988, Kang and Nienstaedt 1987). While any one population may loose specific alleles to random drift (the effect of sampling) or selection, each population will lose different alleles. As a result, each population may end up with a different set of genes that correspond to different "adaptive peaks" as defined by Wright (1977).

Examples of breeding programs using the nucleus breeding strategy include the *Pinus taeda* program of the NSCU-Industry Tree Breeding Cooperative (McKeand and Bridgwater 1998), the Southern Tree Breeding Association's *Pinus radiata* program (White et al. 1999) and the New Zealand Radiata Pine Breeding Cooperative (Jayawickrama and Carson 2000). The New Zealand program has integrated aspects of multiple population breeding by having multiple nucleus populations, each emphasizing different combinations of traits. A number of *Pinus patula* breeding programs in southern Africa are using multiple breeding population strategies (Barnes 1994, Dvorak 1997). The second-generation Douglas-fir breeding programs of the Northwest Tree Improvement Cooperative are also utilizing aspects of the multiple population breeding strategy (Johnson 1998). In each breeding zone, selections are only mated with individuals from their local area. Thus, while the same traits are being selected upon, different gene combinations may be selected in each breeding group, since each population comes from different areas and there is clinal variation in adaptive traits.

Selection and mating procedures

Genetic gain is increased in the short term by increasing selection intensity, i.e. choosing only the very best. The drawback to this is that it ultimately decreases the maximum gain in the long term because some favorable alleles are lost to random drift. It also leads to inbreeding depression. However, for a given level of genetic gain, there can be a number of different selection choices, some of which maintain genetic variation better than others. Lindgren and Mullin (1997) and Zheng et al. (1997) present algorithms to maximize genetic gain when inbreeding (reduction in Ne) is given a negative weight. Kerr et al. (1998) expands the idea to maximize gain over many generations (instead of only one generation as done previously) and examines optimum mating algorithms. These methods are more complex than simply limiting the number of selections per family, but will lead to more gain for a given loss of genetic diversity.

King and Johnson (1993) used computer simulation to examine gains and effective populations sizes from a number of mating designs. They limited the number of selections per family as a means of controlling inbreeding. They found that by increasing the number of crosses per selection (i.e., increasing family number), they were able to maintain a higher Ne for a given amount of gain.

Summary

Most breeding programs have sufficiently large breeding populations to maintain rates of gain for up to ten generations for current traits of interest. As new traits of interest arise, additional populations may be needed, since many low frequency genes will not be in the breeding population. Organizations should monitor the existing *in situ* reserves and when necessary, develop *ex situ* populations, preferably in cooperation with other interested organizations.

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