SPECIES AND RACIAL HYBRIDIZATION IN FOREST GENETICS

by H. B. Kriebel 1/

Definition of Terms

A species may be defined as a group of actually or potentially interbreeding natural populations, reproductively isolated from other such groups (Mayr, 1942). Two types of species are recognized:

1. Allopatric, occupying separate geographical or ecological areas. Examples of this type of species are Engelmann spruce and Sitka spruce.

2. Sympatric, occupying the same or overlapping areas. Examples of this type of species are red and white spruces in northern New England.

A race is a population within a species, having general similarities and distinct from other populations, but not enough to be considered a taxonomic subdivision. An example might be Black Hills ponderosa pine.

Hybridization as a broad term refers to natural or man-made crosses of individuals of unlike genetic constitution. In discussing hybridization we are generally considering species or races. An illustration of species hybridization would be the cross of eastern white pine and western white pine. An example of racial hybridization might be a cross of New Mexico ponderosa pine and Black Hills ponderosa pine.

The Relation of Evolution to Hybridization

To understand the process of hybridization one must have some understanding of evolution. The genetic constitution of populations is constantly changing. New genes are introduced through mutation, and genotypes that survive are those best adapted to the locality. Natural hybridization plays a very important role in evolution. According to Anderson (1953) "strong evidence has been presented for the view that the most significant source of presently observed variation in many instances investigated is hybridization rather than recent mutation."

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Studies of crossability patterns of particular genera, such as the one made by Duffield (1952) for the hard pines and Wright's (1955) study of the spruces, lead to hypotheses on evolution, species formation, and taxonomic relationships. This information is basic to scientific tree breeding.

Some Practical Objectives of Hybridization

(a) A tree species valuable for its timber characteristics, but lacking in resistance to a potent disease or insect pest, may be combined with a closely related resistant species to produce a hybrid including all the desirable characteristics. The well-known hybrid of European and Japanese larch, for instance, shows resistance to the larch canker to which its European parent is highly susceptible. The hybrid between European Populus tremula x North American P. tremuloides shows resistance to rust and aspen scab.

(b)1 A species desirable for a certain region from a forestry standpoint but lacking in winter or summer hardiness may be combined with a hardy species to produce a hybrid adaptable to the region. For example, Piatnitsky (1954) reported a drought-resistant hybrid, Caucasian oak x English oak. The hybrid has 30 to 40 percent less water loss in transpiration than English oak. A cross of pitch x loblolly pine is noticeably less susceptible to low temperature injury in 'southern Illinois than is loblolly pine, has reasonably good form, and may have possibilities north of the area to which loblolly itself can be extended (Lorenz and Spaeth, 1953). This hybrid is also being used extensively in South Korea where the climate is too cold for loblolly pine (Hyun, 1956).

(c) Two related but geographically or ecologically isolated species may, when crossed, yield a progeny showing hybrid vigor (i.e., greater vigor than either of the parent species when grown in the same locality). A number of hybrids of European and North American poplars and aspens show this characteristic of heterosis or hybrid vigor (Schreiner, 1959). Another illustration is the hybrid western white pine x eastern white pine.

Two Methods for Production of Hybrid Seed in Commercially Usable Quantities

(a) Direct utilization of F1 or first-generation hybrid seed produced by hand-controlled pollination is possible if viable seed yields are fairly high, or by open pollination if trees are isolated from others of the same species.

(b) Alternatively, the F_1 generation may be utilized as an intermediate for the production of F2 generation hybrids in seed orchards by open pollination or by controlled pollination, often through a backcross with one of the parent species.

The Korean program of mass production of hybrids of the pitch x loblolly cross is an illustration of the direct utilization of the F1 hybrid. In 1956 over 23,000 pollinations were made. It is estimated that the cost of the trees is only about one-third more than that of regular Scotch pine transplants (Hyun, 1956). Another example is the widespread outplanting of the Dunkeld larch.

Although the F1 generation hybrids are fairly uniform, the F2 generation yields a wide range of genotypes and phenotypes. If we take a hypothetical example of simple dominance such as that presented by Langner (1952) in which three phenotypic characters are under study, the F_2 generation will segregate into 27 genotypes. Only one out of 64 trees will be homozygous for all three desirable characters. On the other hand, there will only be 8 phenotypes, and 27 out of 64 trees or 42 percent will have the combination of all three desirable phenotypic characteristics. For a forest plantation in which thinnings are to be made, this is ample. This practical type of approach is being used at the Western Institute of Forest Genetics at Placerville where a seed orchard of lodgepole-jack pine hybrids has been producing low-cost F_2 hybrid seed for several years; the seedlings look promising as a rapid pulpwood producer (Duffield, 1954).

Second-generation hybrids may be produced by backcrossing, that is, crossing a hybrid to one of its parental types. This technique is useful in transferring single characters such as insect resistance to non-resistant but valuable stock. For example, the Jeffrey pine x (Jeffrey pine x Coulter pine) backcross combines the weevil resistance of Coulter pine with the desirable wood quality, form, and vigor of Jeffrey pine (Libby, 1958).

Second-generation crossing may involve a third species. One use is to bridge an incompatibility between two species. Holst (1957) is using this method in spruce breeding. Norway spruce is a desirable species for eastern Canada, but is not resistant to the white pine weevil, as is white spruce. Crosses between Norway and white spruces have not been successful. However, by crossing Norway spruce with another species such as Sitka spruce, and later crossing the hybrid with white spruce, it is hoped that a superior resistant type may be obtained. Another use is to introduce new genes to improve an existent hybrid. This has been done in chestnut breeding. The Japanese chestnut x American chestnut hybrid has fairly good form, but its Japanese parent is only partially blight resistant. The high degree of blight resistance of the Chinese chestnut can be combined by crossing it with this hybrid. Second-generation Chinese x (Japanese x American) hybrids in Connecticut look very promising, attaining 40 feet in height at 16 years with good form and a high degree of blight resistance (Nienstaedt and Graves, 1955).

Influence of Biology and Economics on the Selection of Breeding Material

It is important to know the biological and economic characteristics of the species in the genus, such as site adaptability. Black and red spruces, for example, differ in relative toleration of wet or dry sites. Branch habit and tree form may vary greatly from one species to another, as in the case of Japanese red pine versus Japanese black pine. There may be wide variation in disease resistance, pulping qualities, and other characters.

The degree of gain possible by using species hybridization in addition to race and individual selection depends upon the particular characters for which selection is being made. Such characters as fiber length and specific gravity show considerable intraspecific, in fact individual, variability among the southern pines. In this case improvement is possible by selection, propagation, and the establishment of seed orchards. Again, in sugar maple wide variability in sugar content of the sap is indicated, whereas no other species of maple approaches Acer saccharum in this respect. Here again breeding of individual selections rather than species crossing is the best approach. On the other hand, in eastern white pine there is a relatively slight variability in genetic capacity to resist white pine weevil attack. If any improvement is made it will probably be through hybridization with a more resistant species, possibly Balkan pine.

Interracial crosses may be useful when individual variability within a region is only moderate but pronounced regional differences exist. If a sufficient period of gene isolation has elapsed, hybrid vigor may be obtained. Helge Johnsson (1956) reported heterosis in European aspen crosses using parents from different latitudes. He advocated seed orchards constructed of clones from rather large areas, making it possible to use the seed from the orchard over a wide area. The positive effects of heterosis counterbalance any negative effects of moving provenances. Nilsson (1958) reported very vigorous crosses of Swedish and continental Norway spruce. These interracial hybrids are superior to Swedish spruce in growth, and superior or equal in growth to continental spruce and more resistant to frosts. According to Wettstein (1958), investigations of characteristics of individual races of Scotch pine show the possibility of selective genotype breeding for certain desired characteristics such as form of tree, rate of growth, resistance to disease and cold, shade tolerance, and weight of seed.

Influence of Geographic Origin of Parents and of Planting Location of Progeny on Hybrid Performance

The seed source of parent trees may vitally affect hybrid performance. An excellent illustration at Placerville is the relatively slow growth of the hybrid western white pine of California origin x Himalayan pine in comparison with the immediately adjacent hybrid western white pine of Washington origin x Himalayan pine (Duffield and Snyder, 1958). Of the numerous crosses between European aspen and quaking aspen made in Denmark, the most promising so far seems to be the hybrid between European aspen from Poland and quaking aspen from the district around Vancouver, British Columbia, raised with material from populations cultivated in Denmark (Larsen, 1956).

Consideration must also be given to the region in which the hybrid will be used. In the case of the hybrid western white pine x eastern white pine, plantations in the Western States show hybrid vigor, but less satisfactory results have been obtained in Wisconsin. Langner (1951) states that "the highest growth capacity of hybrids can be expected when pure species from spatially isolated, climatically similar regions are crossed with one another and the hybrids are grown in this same sort of climate. If one of these requirements is not met, the effect of limiting factors is expressed, whereby growth of the hybrid is held down to a level lower than that of the parents."

Individual selection and tree breeding must go together. Obviously although a particular species cross may have hybrid vigor or disease resistance, if one of the parents is badly forked the loss may outweigh the gain. Likewise carefully selected specimens may be expected to produce better hybrid progeny than run-of-the-mill trees. Certain types of selection are unavoidable, such as selection for fruitfulness. Sex of the tree may be important; in dioecious species such as poplars, reciprocal crossing would obviously be impossible unless both male and female trees were available.

Inbreeding

Inbreeding consists of crossing a tree with itself or another individual of identical genetic constitution if it is clonal material. There is great variability in self-fertility of forest trees. Among the maples, some seem to be self-fertile and some self-sterile (Piatnitsky, 1934).

Pines are quite variable. Self-sterility is high in Japanese red pine and low in Japanese black pine, for example (Toyama, 1950). The degree of self-fertility may also be quite variable among individuals of a single tree species, as is the case in Douglas-fir (Orr-Ewing, 1954).

Inbreeding depression is common in many species; western white pine seedlings provide an excellent illustration of this effect (Bingham and Squillace, 1955). Juvenile depression in some character such as height growth may not necessarily follow inbreeding, however. The writer has found no clear evidence of such an effect in sugar maple. In general, the closer the character is related to fitness, the more it is subject to inbreeding depression (Lerner, 1958).

Inbreeding may be used for later outcrossing. In England, both European and Japanese larches are being inbred for later species crossing, with the expectation of obtaining a pronounced heterotic effect in the hybrids (Matthews, 1955).

Obviously, a knowledge of degree of self-sterility is important in controlled pollination work and establishment of seed orchards. The possibility of selective fertilization also needs to be explored. A tree may be self-fertile when artificially pollinated, but under natural crossing its own pollen may be discriminated against in favor of pollen from other trees (Squillace and Bingham, 1958).

Breeding Technique

Breeding technique includes at the outset collection, extraction, and storage of pollen. Flowers may be collected from trees in the field, picked catkins, branches forced indoors, or even branches grafted in the greenhouse. In some cases, as in poplars and elms, the seed can be produced on cut branches.

Pollen extraction techniques range from flat sheets of paper to the elaborate extractors developed at Placerville (Cumming and Righter, 1948). One simple type is a sleeve of sausage casing. Another is a small airtight box with removable screen gauze and stopper funnel at the bottom.

Pollen storage techniques vary with the species. Pine pollen can be stored at 25- to 50-percent relative humidity at 38 to 40 degrees F. (Duffield, 1953). Deep-freeze techniques may prove more satisfactory for long-term pollen storage. Year-old pollen often gives high seed yields but a low percentage of viable seeds (Wright, 1959). However, at times it is necessary to use year-old pollen, as in pollination of pines in Ohio with pollen from western soft pines (limber, sugar, western white, and whitebark pines) on which pollen is late in maturing.

Flowers are isolated by bagging to prevent random open pollination. Observation of the rate of development of flowers is of the utmost importance to the success of controlled pollination. Pollen must be applied at the time the flowers are open and receptive, although our present knowledge of duration of period of receptivity is quite limited for many species. While much valuable data lie buried in tree breeders' notebooks, relatively little has been published. Detailed information of this type has only recently been published on Scotch pine (Ehrenberg and Simak, 1957), although this is one of the world's most intensively studied tree species.

The method of pollen application will depend on the mode of pollen transport. Pollen can be brushed on with a small brush or with the male flowers themselves in the case of an insect-pollinated species. If the species is wind-pollinated, pollen is usually blown into the bag by means of a rubber bulb and hypodermic needle. After the pollination period is over, the bag is generally removed, although it may be kept on, or another bag put on, for protection against insects.

Careful labelling and recordkeeping are essential. A soft aluminum tag with paper backing and copper wire is useful; it can be numbered in advance and placed in the bag with a wad of non-absorbent cotton to be wrapped around the shoot at the mouth of the bag. A cloth streamer is often attached to improve visibility. Records are kept of every pollination; a simple type of record sheet adaptable to a pocket notebook is illustrated in U.S. Dept. Agr. Cir. 792, by Cumming and Righter (1948).

Seed collection and seed handling are time-consuming operations because of the meticulous care required to retain the separate identity of each seed lot.

Evaluation of Hybridity

Identification of hybrids, both natural and artificial, consists of grading the putative hybrids for physical characteristics of seeds, cones, twigs, and leaves; study may include the internal anatomy of the leaves; also their color, pubescence and number and position of stomata. Points of interest are not only taxonomic characters but also such characters as resin chemistry in pines (Mirov, 1942, 1946).

Size measurements may be taken of leaves and buds, and analyses made of the number and degree of appression of bud scales. Comparisons may be made with parental physiological responses such as growth phenology, winter hardiness, and drought resistance.

The number of characters requiring scoring in a hybrid index depends on the reliability of the individual characters, and may range from one to several dozen. Zobel (1951) used 12 characters for evaluating natural hybridization between Coulter and Jeffrey pines, including cone, seed, foliage and oleoresin characters. In other work with pines, effective discrimination of F_1 and F2 hybrids of knobcone and Monterey pines is possible using seven characters (Bannister, 1958). Recently, Schfltt and Hattemer (1959) reported that analysis of pine hybrids solely by means of the characters seen in transverse needle sections is insufficient. Mergen (1959) has recently reported on studies indicating that stomate distribution is a reliable criterion of pine hybridity.

Polyploidy

Nilsson-Ehle's discovery of the vigorous triploid aspen in 1935 (Nilsson-Ehle, 1936) aroused interest in the use of polyploids in tree breeding. The triploids were 11 percent taller, 10 percent larger in diameter, and 36 percent higher in volume than adjacent diploid aspens of the same age. Triploids are generally sterile, but occasionally crosses of diploids and triploids yield tetraploids, permitting quantity production of triploids.

Examples of polyploids are the natural triploid of European white birch (Johnsson, 1944), natural tetraploids of Norway spruce in Sweden (Kiellander, 1950), tetraploid European larch, and tetraploid Caucasian alder (Larsen, 1956).

Polyploids can be used in hybridization. The diploid Italian alder x tetraploid Caucasian alder has vastly greater growth than open-pollinated Italian alder. Extraordinary vigor was shown by a triploid hybrid from a controlled pollination of European larch with western larch. Natural polyploid hybrids have been discovered, as in the case of the natural triploid hybrid of *Betula* pubescens and *Betula pendula*, discovered by Helms and Jorgensen (Larsen, 1956).

Conclusions

1. Hybridization of species and races is a natural process of evolution that is continually occurring in forest trees as a .result of climatic changes and other factors promoting changes in gene distribution.

2. Heterosis or hybrid vigor is fairly common among tree crosses of species from climatically similar but geographically isolated regions, and has its greatest expression when hybrids are planted in climatic regions similar to those of the parents. If climates of the parent species are different, race research is particularly important to make the best crosses for use in particular regions.

3. Inherent tendency to withstand or succumb to diseases and insect pests not infrequently is common to an entire species or race, by virtue of long-term previous exposure to or isolation from these injurious agencies.

4. Species or racial hybridization may be the most effective technique for tree improvement in such cases, by the combining of genes for resistance with genes for other desirable characters.

5. Hybridization is most effectively used in connection with selection - not only race selection, but careful selection of superior individual specimens from each of the parent species.

6. Finally, hybridization is already beginning to be used for mass production of superior tree types both in this country and abroad. There is every reason to believe that it will play an increasing part in scientific forest management.

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