Reprinted from
Proc. Fourteenth South. For. Tree Improv. Conf.
[Gainesville, Fla., June 1977.] p. 239-245.

USE OF MUTANTS IN FOREST GENETICS RESEARCH

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Abstract.-- Numerous genetic abnormalities (mutants) affecting pigmentation, development, and form have been described for the southern pines. Many of these traits behave as simply inherited Mendelian traits when produced by selfing and crossing. Such traits can often be used as genetic markers to trace pollen distribution, to test relative effectiveness of various pollens, or to make inferences about the genetic structure of stands or other populations such as seed orchards.

Additional keywords: Mendelian traits, particulate inheritance, pollen distribution, genetic structure of populations, seed orchards.

POPULATION GENETICS

Study of population genetics is directed toward descriptions of defined biological populations in (1) their present genetic structure, and (2) trends through time of gene frequencies, means, variances, and distributions of quantitative traits. Based on the above description, a quantitative trait may be defined as one influenced by the combined action of many genes, each with small effects relative to the observed variability of the trait. The analysis of such a continuously variable trait--height growth, for example--requires the use of statistically descriptive units such as means and variances. Such traits are said to be subject to the laws of quantitative inheritance. Since the effects of individual genes cannot be detected, gene frequencies and distributions cannot be determined.

Qualitative traits are categorized at the opposite end of the spectrum as those which can be uniquely described in terms of color, texture, size, migration distance, etc. Such traits are subject to the laws of particulate (rather than quantitative) inheritance because each variant can be related to a particular gene. This is also called Mendelian inheritance in honor of Gregor Mendel, who first described such inheritance on the basis of his classical experiments with garden peas. The utility of rare mutant genes in studies of population genetics of trees is based on the exactness of Mendelian inheritance, which permits prediction and interpretation of patterns and frequencies of mutants under specified hypothetical conditions.

ALLELIC BASIS OF MENDELIAN INHERITANCE

Chromosomes are the physical structures within the nucleus of a cell which contain most of the chemically encoded genetic information necessary for development and maintenance of a tree. Chromosomes occur in pairs; for example, pines have 12 pairs or a total of 24 chromosomes (Mirov 1967). As the term is commonly used, a gene is a genetic regulator which is located at a particular locus (place) on the chromosome. The chromosome consists of a long

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series of such loci arranged in definite linear order. Each member of a pair of chromosomes contains the same number and unique order of chemically encoded genes, each at its own locus. Thus, it is proper to say that the gene for a Mendelian trait is carried on a particular chromosome and that each ordinary cell of the tree will carry two genes for that trait.

The chemical arrangement (base pairing) of four simple organic molecules, called nucleic acids, determines the genetic information contained by a gene. This chemically coded genetic information is called an allele. The average gene contains 1,500 base pairs. By a change in base pairing, an allele can be altered chemically and changed from one code to another. Thus, the average gene could have 4^{1500} different alleles (Watson 1965). When the result of one such chemical change is seen in a tree, that result is called a mutation, i. e., a change in the chemical code of the gene forming a new allele which causes a distinct difference in the tree. The allele which causes the organism to appear "normal" is called the "wild type" and has the highest frequency in a natural population. Other alleles at the same locus are called mutants, and by definition are lower in frequency (rare) and do not produce the "normal" tree.

If a tree contains a wild type allele on one chromosome and a mutant type on the other, it will usually appear to be normal, just as though it had wild type alleles on both chromosomes. This property of the wild type allele is called complete dominance. Mutant alleles which are hidden by the wild type through dominance are called recessive alleles, and they appear in differing but predictable ratios (segregation ratios) when trees are crossed or selfed. In this way, mutants can be discovered, described, and evaluated for future use as genetic marker genes in forest genetics research (Franklin 1970).

DESCRIPTIONS OF MUTANTS

Morphological Description

Some of the most striking mutants in pine seedlings and trees are chlorophyll deficiencies of various types (Snyder et al. 1966, Franklin 1969). Color of the cotyledons in these mutants ranges from pure white, yellow, and yellow-green to pale green. Numerous other foliar variations are found including forms that change color through development (Kraus and Squillace 1964), repeat a color change annually, or show special coloration only under certain environments. Hypocotyls, primary and secondary needles, and even conelets and pollen catkins (Johnson and Critchfield 1974) may show color variants because of recessive alleles. Distinct variations in seedling and tree size and form, length of needles, and other morphological abnormalities have been described (Franklin 1970). There are so many possible alleles for each trait that the possibilities for variation are almost infinite.

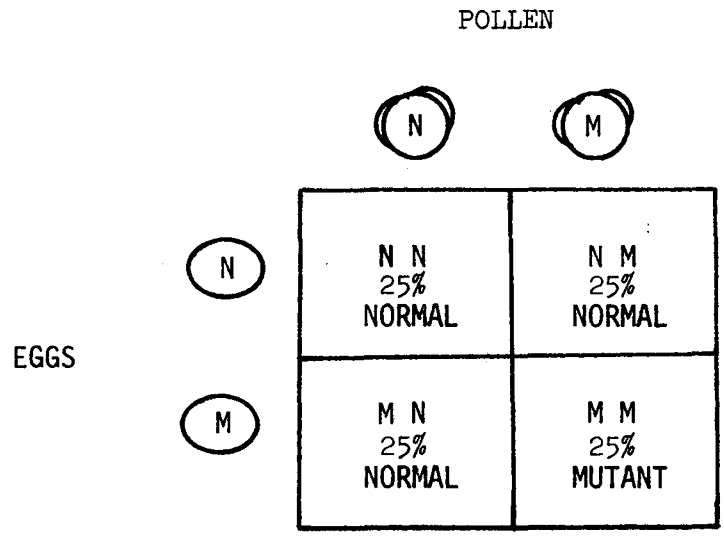
Statistical Description

Statistical data such as segregation ratios and yields of filled seed after selfing and crossing, as well as frequency of the mutant alleles in the population, are necessary to assess the potential usefulness of a particular marker gene. A marker gene is one that has one or more mutant alleles which are suitable for interpreting patterns of gene transfer and assortment through a suxual generation within and between trees or within and between populations.

Self-fertilization is one of the most efficient ways to discover and describe mutations. In loblolly pine, about one tree in four will show a distinct segregation pattern for at least one readily discernable recessive mutant allele causing abnormal coloration or form early in seedling development (Franklin 1969). The procedure is to self- and cross-pollinate several trees in a population of interest--for example, a seed orchard. When seed is collected and extracted, careful records must be kept on yields of filled and empty seeds. One effect of selfing is a large reduction in yield of filled seed in comparison with yields after crossing.

Seeds should then be sown in a greenhouse or other area of relatively moderate and controllable environment. Harsher environments such as a nursery result in fewer mutants being discovered and reliably described. This result occurs because of a combination of environmental effects on both the seedlings and the investigator. Careful observations and counts must be made on all seedlings, especially those which appear abnormal in any way. Once a mutant type can be confidently identified, frequency data can be used to estimate segregation ratios of mutant to normal types.

Under ideal circumstances, a tree containing one dominant wild type allele and one recessive mutant allele will yield 25 percent mutant offspring, 75 percent normal offspring when selfed. Since half of the pollen and half of the eggs contain the mutant allele, the probability that an individual has two normal alleles or two mutant alleles or one of each type is the product of the frequencies of those alleles (fig. 1). Chromosomal linkages frequently exist between mutant alleles under study and embryonic lethals



EGGS \ POLLEN	N	M
N	N N 25% NORMAL	N M 25% NORMAL
M	M N 25% NORMAL	M M 25% MUTANT

Figure 1.-- When a tree having one normal allele (N) and one mutant allele (M) is selfed, the result under ideal circumstances is equal frequencies of four types of offspring (genotypes), 75 percent of which are normal due to dominance of the normal allele and 25 percent of which are the mutant form.

(alleles which cause death of the embryo). In this case, segregation ratios may be distorted from the expected 3 to 1 ratio of normal to mutant types (Sorensen 1967). Therefore, the segregation ratio for each tree under selfing or crossing with another tree must be determined and confirmed before those alleles can be utilized as genetic markers. When chromosomal linkages cause simple Mendelian mutants to show up more frequently in a family than the expected 25 percent, this is an advantage because it increases the statistical precision of tests for a given number of offspring surveyed (Franklin 1974). On the other hand, if very few mutants appear in a family, prohibitively large numbers of seedlings must be surveyed, and that particular mutant allele is not a useful marker.

USES OF MARKER GENES

Although most mutants are deleterious, some have little noticeable effect on subsequent growth. This type mutant can be very advantageous if the abnormality can be observed early in seedling development. An example of such a mutant was described by Franklin (1969) as "green hypocotyl." As early as one day after germination, seedlings were distinguished as having either a normal (reddish-brown) or a mutant (green) hypocotyl. Beyond the cotylendonary stage, both types seemed to develop normally, including cone and seed production at ages 6 to 7 years. The significance of this type of mutant is that pure lines for the mutant type can be established and maintained by controlled crossing because all mutant types have only the mutant allele. An application of the green hypocotyl mutant for study of pollen contamination was made by Weyerhaeuser Company scientists, who established a small experimental seed orchard of loblolly pine consisting entirely of the green hypocotyl strain. When the orchard produces seed, all orchard-pollinated seedlings will have green hypocotyls, but pollen from the background pollen-contamination load will produce normal "wild type" seedlings. In this situation, the percentage of background contamination will be measured directly by the percentage of normal seedlings produced in the orchard. The fact that the mutant is very rare in the general population permits this direct approach.

Another example of the use of genetically marked pollen was reported by Franklin (1974). It involved a single slash pine tree (G-244) on the Austin Cary Forest, University of Florida, Gainesville. The objective of the study was to determine the effectiveness of the first pollen to reach a receptive strobilus versus the effectiveness of pollens reaching the strobilus 1, 2, 3, or 5 days later. The tree was known to produce progeny after selfing with relatively high and predictable frequencies of albino seedlings. The following controlled pollinations were repeated in two different years with the results being averaged:

1. Self-pollen from G-244.
2. Cross-pollen from a tree known not to carry the albino allele.
3. Mixture of 50 percent self and 50 percent cross pollen.
4. Self followed by cross pollen immediately and after 1-, 2-, 3-, and 5-day intervals.
5. Cross followed by self pollen immediately and after 1-, 2-, 3-, and 5-day intervals.

When self-pollen was applied first, its effectiveness was estimated directly as the percentage of selfing. When cross-pollen was applied first, its effectiveness was estimated as 100 minus the percentage of selfing, i. e., the percentage of crossing. The first pollen applied had an average effectiveness of 67 percent. Results were consistant with the hypothesis that the first pollen to reach a receptive strobilus was the most effective in accomplishing fertilization (table 1). Differences between self and cross pollens are consistent with a second hypothesis that when two or more fertilizations take place within the same ovule, cross-pollinated embryos usually outcompete self-pollinated embryos, resulting in higher yields of cross-fertilized seedlings. Thus, cross-pollen applied first averaged 78 percent, while self-pollen applied first averaged 56 percent effectiveness (table 1).

Table 1.-- Results of a controlled-pollination experiment showing that the first pollen received by receptive conelets was more effective in fertilization than pollens received later (from Franklin 1974).

Type of pollination	Number seedlings screened	Percent albinos	Estimated percent selfing	Percent effectiveness of 1st pollen	Mean percent effectiveness of 1st pollen
Self	264	32.2	100.0	---	---
Cross	456	0	0	---	---
50-50 Mixture	159	15.1	46.9	---	---
Self-cross 0[a]	172	13.4	41.5	41.5	55.9
Cross-self 0	177	9.6	29.8	70.2	
Self-cross 1	285	17.5	54.5	54.5	66.1
Cross-self 1	223	7.2	22.3	77.7	
Self-cross 2	59	20.3	63.2	63.2	74.7
Cross-self 2	68	4.4	13.7	86.3	
Self-cross 3	86	17.4	54.2	54.2	63.5
Cross-self 3	103	8.7	27.1	72.9	
Self-cross 5	234	21.8	67.7	67.7	76.1
Cross-self 5	260	5.0	15.5	84.5	
Means: Self-cross				56.2	67.2
Cross-self				78.3	

a/ "Self-cross" denotes that pollen from the seed parent was applied first, whereas "cross-self" denotes that pollen from another tree was applied first. Numbers 0, 1, 2, 3, and 5 indicate days lapsed between application of 1st and 2nd pollens.

SUMMARY

These two examples of the application of mutant alleles as genetic markers to study genetic structure of populations differ distinctly in complexity as well as kind of information sought. There are many other possible applications, all of which rely on the same basic principles and considerations discussed here. The advantages of using genetic markers are:

1. No expensive or complex analytical equipment is needed.
2. Skills and procedures for crossing and selfing trees, and collecting, processing, and germinating seeds are readily available.
3. Useful mutants for markers are readily available in wild and selected populations.
4. Large populations of seedlings can be screened and scored easily.

Some disadvantages are:

1. Reliable physical and statistical descriptions of the mutant forms are necessary.
2. The relative rarity of most of these alleles limits some application possibilities.
3. Relatively low yields of seed from selfing often prohibit use of otherwise acceptable marker-bearing trees.
4. An 18-month wait is necessary to get control-pollinated seed.

Genetic mutants as marker genes may offer some especially attractive opportunities for research and assessment of patterns of pollen distribution in individual trees (Squillace and Kraus 1963), seed orchards (Franklin 1971, Kraus 1975), or under certain imposed conditions of control-pollination. Even certain inferences about wild stands can be made if population samples are large. The utility of these mutants must be evaluated against alternative methods before the best choice of methods can be made.

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