A POCKET OF VARIABILITY IN *PINUS RIGIDA*

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Steady state gene frequencies around a pocket of differential fitness have been formulated by Hanson (1966) in a generalization of the work of Haldane (1948). A pocket of differential fitness would result in a pocket-of-variability, assuming that the radius of the area of contrasting fitness was large in relation to the vagility of the organism. Conversely, the absence of a pocket-of-variability is predicted whenever dispersal is broad relative to the size of the pocket of differential fitness. Around the pocket, a cline would be established through the interaction of migration and selection. The slope of the cline would depend on the width of the band of intermediate fitness, being steepest where the band was absent. The change in gene frequency with distance from the pocket would be rapid in the band of intermediate fitness values and approach limiting values asymptotically in the areas of contrasting fitness.

The serotinous cone habit in pitch pine (*Pinus rigida* Mill.) provided us an example of a pocket-of-variability. A serotinous cone is one that remains closed for at least one year after maturity. The cone scales are held together with a resin seal and heat is required for prompt opening. Normally, temperatures in the tree crown do not reach levels sufficient to open serotinous cones. Serotinous cones do open and shed seed following a fire, so that serotiny seems to be an adaptation to fire, ensuring reproduction at the most opportune time in relation to site availability.

To properly relate patterns of variability to Hanson's model, characteristics controlled by a single gene should be considered. For Jack pine (*Pinus banksiana* Lamb.) there is evidence that the closed-cone characteristic may be under simple genetic control (Rudolph et al., 1959). Assuming single gene inheritance and no dominance, Teich (1970) demonstrated that populations of both jack pine and lodgepole pine (*Pinus contorta* Dougl.) fit Hardy–Weinberg expectations. The elegant analysis of Sittmann and Tyson (1971) makes it highly probable that inheritance is single-gene and that serotinous-coned trees and open-coned trees are the respective homozygotes while trees with both types of cones are heterozygotes. It seems likely that the serotinous cone habit in all pines is basically under the control of a single locus. With the long generation time of trees, decades may be required for further verification.

In pitch pine some trees bear serotinous cones. Pond pine (*Pinus serotina* Michx.), with a nearly allopatric range to the south of pitch pine, is nearly 100% closed-cone (Fowells, 1965). Pond pine was treated with specific status by Critchfield and Little (1966) but was considered a subspecies of pitch pine by Smouse (1970). On Cape May, New Jersey and on the Delmarva Penninsula a transition type occurs which belongs to neither group, according to Smouse (1970). Little et al. (1967) also reported that these areas were zones of intergradation.

Variation in the frequency of serotinous-coned trees has long been a subject of speculation. It would be reasonable to hypothesize that the variable occurrence of serotinous cones in pitch pine is related to the influence of pond pine through active hybridization and gene flow, and that the frequency of pitch pine with closed cones would decline with distance from Cape May. A second hypothesis is that the distribution of trees with the closed-cone habit is related to some underlying selective factor(s), possibly fire.

Literature Review

Geographic variation in the closed-cone habit has been described in other North American pines. For example, frequency of serotinous cones in lodgepole pine decreased from the Rocky Mountains to the Pacific Coast though there was a great deal of local variation (Critchfield, 1957; Lotan, 1967; 1968; Mowat, 1960). Critchfield (1957) found that the Mendocino White Plains, an area of dwarf vegetation, supported a serotinous-coned race of lodgepole pine while open-coned types were most frequent elsewhere on the Pacific Coast. In jack pine, frequency of the closed-cone habit decreased from north to south (Rudolph et al., 1957; Schoenike et al., 1959). In sand pine (*Pinus clausa* [Engelm.] Vasey) the Choctawhatchee race in western Florida is open-coned while the cones of the Ocala race from peninsular Florida remain closed at maturity (Little and Dorman, 1952). The area in which the Ocala race is native is subjected to crown fires on the average once a generation, while fires are not as severe in the range of the Choctawhatchee race. Therefore, Little and Dorman (1952) suspected that fire ecology was responsible for the difference between the races.

Methods

In October and November, 1969 and 1970, ripe cones were collected throughout the range of pitch pine, extending from Quebec, Canada, south to Georgia and from the East Coast west to central Ohio and Kentucky. Cones were kept separate by individual tree and were placed in a cone drying rack of wire mesh construction. The cones were maintained at a temperature of about 30C. A temperature of 30C is certainly above that normally encountered during seed shed in the fall and should be sufficient to open non-serotinous cones. Though the temperature required for opening serotinous cones in pitch pine has not been determined, in both lodgepole and jack pines it was found to be about 45C (Clements, 1910; Cameron, 1953).

After a few weeks, most cone lots (i.e., collections from individual trees) had opened. Lots which remained closed after several months were considered homozygotes for the serotinous cone locus. The lots with open cones were regarded as open-cone homozygotes or mixed-cone heterozygotes. The procedure is considered valid because Crossley (1956) demonstrated that cones from open-coned lodgepole pine opened readily during air-drying while those from serotinous-coned trees did not open at all under the same conditions. The method of scoring after air drying was preferred to the scoring of serotiny in the field, because in the field it was often difficult to distinguish between truly serotinous cones and those which had failed to open because of minute injury or which had opened and then closed hygroscopically. Smouse (pers. comm.) while investigating introgression in pond pine also had difficulty scoring serotiny in the field and the analysis of Sittmann and Tyson (1971) indicated that substantial misclassification has occurred in previous field studies. In the forest there is danger of misclassifying heterozygotic mixed-cone individuals as either closed-cone or open-cone.

Methods of choosing sample populations of pitch pine and their location will be covered in more detail elsewhere, but were unbiased in relation to the closed-cone habit. Locations were chosen to sample the range systematically. In pitch pine stands near pre-set locations, cone-bearing trees were picked essentially at random, generally at fixed distances along trails, roads, or compass bearings.

In most of the 1969 and 1970 sampling, two stands were chosen to represent each area and cones were collected from six trees in each stand. Because of squirrel or insect predation or accidental loss of trees, cones were collected from less than six trees in a few cases.

The initial sample suggested that the closed-cone habit was restricted to the

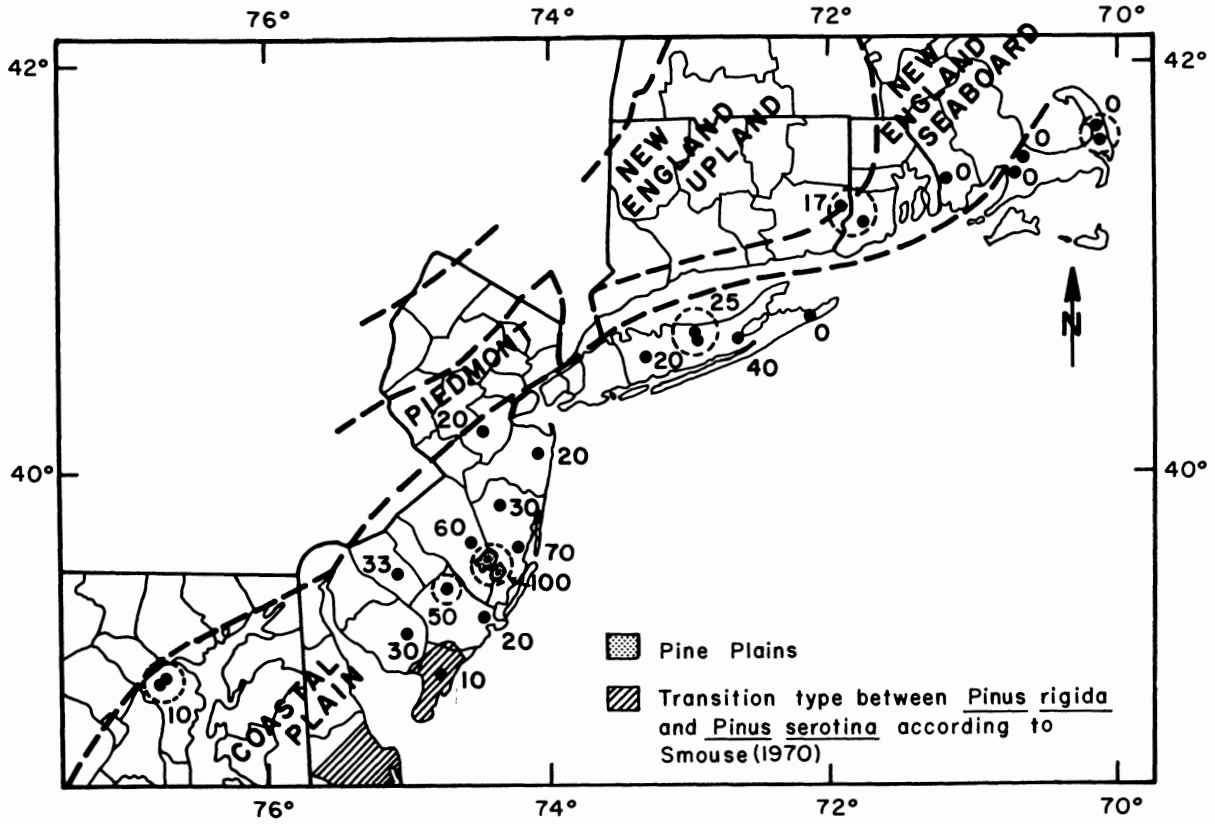

FIG. 1. Map of the Coastal Plain and the New England Seaboard within the range of pitch pine, showing the percentage of trees that were completely closed-cone. Stands encircled with dotted lines were paired stands with 6 trees each (in three cases, only 5); other stands have 10 trees each (in two cases, only 8 and 9). Note the apparent decrease in frequency of the serotinous-cone habit in concentric rings around the New Jersey Pine Plains and the low frequency in the stand located within the transition region between pitch pine and the serotinous-coned pond pine. Physiographic regions after Lull (1968).

Coastal Plain, so additional samples were taken in 1970 to cover this area at about 20 mile intervals. In each of the additional 15 stands, cones were collected from 10 trees except in two cases when only 8 and 9 trees, respectively, were included.

Results and Discussion

The total number of trees represented by cone collections was 509, representing 79 stands. Trees with serotinous cones were very rare in all areas except on or very near the Coastal Plain. Away from the Coastal Plain only nine trees were found to have serotinous cones. Of these, there was one tree each in stands from Steuben Co., N.Y., Augusta Co., Va., and Clay Co., W. Va. The remaining six (100% of those sampled in the stand) were from Clinton Co., Pa. Personal communication from the District Forester in Clinton Co., J. E. Paulhamus, indicated that stands in the latter area originated after extremely severe fires.

Generally, all of the cones of an individual tree either opened or remained closed. That is, as judged by the method used in scoring serotiny, there were almost no intermediates between trees classed as serotinous vs. non-serotinous. Only five trees had both closed and open cones. Frequency for the serotinous cone gene was calculated as the square root of the proportion of trees having only closed-cones. Individual lots collected in both 1969 and 1970 behaved identically in both years, suggesting that penetrance is complete.

Between stands chosen to represent the same location, there was occasionally substantial variation. At Brookhaven National Laboratory, Long Island there were no trees with serotinous cones in one stand, but in another stand about four miles distant, 3 of 6, or 50% of the sampled trees had serotinous cones. Though the samples are small, the frequency of closed-cone trees in the sample always agreed closely with field estimates of the frequency.

The frequency of trees with serotinous cones did not show a maximum nearest the range of pond pine which is nearly 100% serotinous-coned, (or nearest the transitional type between pitch and pond) with declining frequencies northward as would be expected if introgression and migration were the only forces influencing the distribution of the characteristic (Fig. 1). It is notable that in the Cape May area from which the serotinous-coned pond pine has been reported, and whose population Smouse (1970) classifies as a transition type, the frequency of serotinous-coned trees in the sample was very low (10%). Because it is based on only 10 trees, there can be little confidence in the estimate; however, it fits well into the statistically significant pattern reported below. Clausen (1939) also considered the Cape May population as a true intermediate between pitch and pond pine. Thus, it would seem that in this characteristic, pitch pine does not grade toward pond pine.

Rather, there seemed to be a decrease in the frequency of serotinous-coned trees in all directions from the New Jersey Pine Plains. The Pine Plains are areas of dwarf forest comprising up to 20,000 acres within the New Jersey Pine Barrens. A point was marked midway between the two areas known as the West and East Plains and distances from this point to other stands were scaled. Frequency of the gene for serotinous cones seemed to drop off logarithmically with distance from the point (Fig. 2). Results are virtually the same whether gene frequency or frequency of trees bearing serotinous cones are considered. The correlation between the arcsin $\sqrt{\text{per cent}}$ transformation of the gene frequency and the logarithm of the distance of the stand from the Pine Plains was $r = -0.86$ ($P < .001$). In the analysis, the value $100/4n$ (where n is the number of trees in the sample) was substituted for 0% and $100 - 100/4n$ for 100% (Steel and Torrie, 1960, p. 158).

The gradation from a high frequency for the serotinous cone habit in the Pine Plains to a zero frequency in distant areas could reflect a difference in selective factors. In the Pine Plains proper, all trees (100%) in

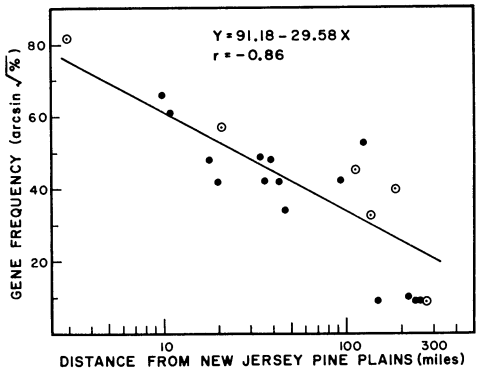

FIG. 2. Decrease in gene frequency (Y = arcsin $\sqrt{\text{percent}}$ transformation) with distance (X = $\log_{10}$ distance) from the New Jersey Pine Plains. Closed circles (●) are 10-tree stands, open circles (⊙) are combined values for two paired stands of 6 trees each (in some cases points are based on fewer than the nominal number of trees).

the sample were of the serotinous type. Open-coned trees are present but are rare (up to 2% in some areas according to personal communication from S. Little). The dwarf habit of pitch pine in the Plains, generally 6 to 8 feet in height, has been attributed to fires which are more frequent and severe than in the surrounding area (Lutz, 1934; Andresen, 1959).

If the serotinous cone habit is directly related to the occurrence of fire, then the frequency or severity of fires must decrease in all directions from the Pine Plains but most rapidly with distance inland from the Coastal Plain. No quantitative data is available on this point, but old records and newspaper accounts reviewed by Lutz (1934) suggest that the area in and adjacent to the Pine Plains has been more frequently and severely burned than the surrounding Pine Barrens. The age attained by trees in South Jersey is determined by the frequency of intense fires. Lutz (1934) placed the frequency of fires at 16 to 26 years in the Pine Barrens, decreasing to 6 to 8 years in the central Pine Plains. Moreover, because of the low height of the Pine Plains vegetation, fires there are more severe, each one being a crown fire. Such studies of fire ecology suggest that the frequency of the serotinous cone gene may reflect an underlying pattern of fire history. Serotinous cones are adapted to shed seed after exposure to high temperatures, resulting in high levels of reproduction on sites opened to colonization by fire. The consequences of not shedding seed may be failure to reproduce once the fire zone is passed, and selection against serotinous-coned trees would be high where fires are infrequent.

The geographic pattern observed, rapid initial decline in gene frequency from a pocket and a slow decline to zero peripherally, resembles theoretical curves of equilibrium gene frequencies in relation to distance from the center of an environmental pocket of differential fitness (Hanson, 1966). The pattern suggests relatively narrow zones of neutral or intermediate fitness of the serotinous cone habit between a pocket in which its reproductive value is very high and peripheral regions where it is nil or close to it. The cline in frequency of the serotinous cone characteristic need not reflect a gradual and continuous pattern of variation in selective factors. Similar clines can be established through the action of migration between two areas of sharply contrasting fitness (Hanson, 1966).

An alternative hypothesis that the pocket is transitory, rather than steady state, is considered unlikely. Pitch pine forms a continuous population over the nearly 2000 square miles of the Pine Barrens and its distribution is believed to be an ancient one (Harshberger, 1916). There is no evidence of previous isolation for the Pine Plains.

If fire is responsible for the selection of serotinous-coned trees, then the occurrence of predominantly open-coned pitch pine on frequently-burned glacial outwash plains must be explained. An example of a frequently-burned local area is the Albany–Schenectady Sand Plain where repeated fires have been so severe that most stands have a devastated appearance. However, none of the trees in the sample from this area had serotinous cones. In this case, the

pocket may not have been broad enough to sustain the closed-cone habit in the face of migration from surrounding areas. Hanson (1966) suggested that environmental pockets of relatively small size in relation to dispersal distances may be completely obscured by the effects of migration, and that in pine, pockets of 15–20 miles in diameter may be too small to create pockets-of-variability. Pine seed is capable of transport to distances of 11 miles (Bannister, 1965) and pollen dispersal in conifers may cover many scores of miles under proper atmospheric conditions (Lanner, 1966). Perhaps, outwash plains are too small to create pockets-of-variability. Thus, theory and observation suggest that high rates of fire occurrence may not always result in high frequency of the closed-cone habit.

Levins' (1968) models may also explain the variable occurrence of serotinous or non-serotinous cones. The closed-cone habit and the open-cone habit are two points which define a concave fitness set. Fire would be a coarse-grained feature of the environment. Selection would result in the existence of both cone types in the same population, in a proportion which depended on the environmental pattern. However, the absence of serotinous-coned trees in many areas which have a fire history and the regular decrease in the frequency of the serotinous-cone habit with distance from a central point, suggest the importance of migration. The data tend to fit the models of Hanson (1966) better than those of Levins (1968), indicating a balance between the forces of selection and migration.

Summary and Conclusions

The frequency of serotinous-coned pitch pine is highest in the New Jersey Pine Plains, an area of dwarf vegetation, and the immediately surrounding area in the Pine Barrens. There is evidence that serotinous-coned trees are homozygotes for a single gene controlling the trait. Under this assumption, gene frequency declines rapidly to zero within at least 200 miles in all directions, decreasing logarithmically as the distance from the Pine Plains increases ($r = -0.86$). The distribution suggests a very high selective advantage for serotinous-coned trees in the vicinity of the Pine Plains and a selective disadvantage for the trait away from this area, creating a pocket of variability. A steady state pocket-of-variability is considered more likely than a transitory pocket.

The factors important in the establishment and maintenance of the cline are probably 1) an environmental pattern of fire occurrence and severity and 2) gene flow through pollen and seed dispersal. Serotinous cones open after fires and are advantageous in recolonizing a site following severe burns. However, the consequences of not releasing seed would be disadvantageous beyond the fire zone. Gene migration would result in clinal patterns of variation, and because pine has a high dispersal potential, migration might be considerable, even obscuring smaller pockets of differential fitness as predicted by Hanson (1966).

The alternative hypothesis that the occurrence of serotinous cones in pitch pine is directly related to active intergradation from the serotinous-coned pond pine taxon is rejected. The frequency of serotinous-coned trees is low in the transition area between the two taxa. The gene for serotinous cones may have originated in pond pine and been transferred to pitch pine by hybridization during Pleistocene glaciation. The high selection coefficients hypothetically associated with the serotinous cone habit would obscure evidence of current intergradation in the characteristic.

The results are of primary interest as an example fitting Hanson's (1966) model of steady state frequencies between pockets of differential fitness. The models predict a cline (with a logarithmic gradient) around a pocket-of-variability and also predict the absence of pockets-of-variability when the pocket of differential fitness is too small. The similarity of the observed pattern of variation to the theoretical suggests a ma-

jor role for migration in the microevolution of forest trees.

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