

## Genetic aspects of fertility differentials in ponderosa pine

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### SUMMARY

There is a strong genetic component to fertility differentials among individuals of ponderosa pine. Prolific cone-producers as a group were markedly different from low cone-producers at the three protein loci which were monitored. The two groups did not differ significantly in age, but trees with high cone production had slower growth rates and smaller diameters than trees with low cone production. To our knowledge, these results provide the first demonstration of fertility differentials associated with specific genes in a woody plant.

### 1. INTRODUCTION

The differential reproduction of genotypes is a primary driving mechanism of natural selection. Yet studies of selection in natural populations seldom focus upon fertility differentials (Christiansen & Fenchel, 1977). This is particularly true in studies of differential reproduction in plant populations. Differences between phenotypes are acknowledged to have a probable genetic component, but the factors considered to be important are usually plant density, size, age, vigour or vegetative growth (Stern & Roche, 1974; Dorman, 1976; Harper, 1977). Agriculturists, using artificial selection, have repeatedly demonstrated that various components of plant reproduction are under genetic control (Allard, 1960; Brewbaker, 1964). However, to our knowledge, there are only four precedents in the literature for associations of fertility differentials and specific protein genotypes in natural populations. Both viability and fertility differentials were reported for protein genotypes of barley, *Hordeum vulgare* (Clegg, Kahler & Allard, 1978) and the slender wild oat, *Avena barbata* (Clegg & Allard, 1973). Flowering individuals of the composite *Liatris cylindracea* were more likely to be heterozygous than were non-flowering individuals (Schaal & Levin, 1976), and genotypes of the milkweed bug, *Tetraopes tetraophthalmus*, captured while copulating were different from those collected singly (Eanes *et al.* 1977). It has also been reported that there is no association between fertility and randomly chosen

protein genotypes in the eelpout, *Zoarces viviparus* (Christiansen & Frydenberg, 1976).

## 2. MATERIALS AND METHODS

The population of *Pinus ponderosa* var. *scopulorum* analysed here consisted of 217 trees distributed over an area of about two hectares. The population is located at approximately 1740 m elevation on the south-facing slope of Boulder Canyon, Boulder County, Colorado. Its low density and age structure are typical of mature stands of ponderosa pine in this region. The 217 individuals studied were all 33 or more years old and 4 m or more tall and were considered old enough to reproduce. An additional 22 seedlings or saplings, not included in this analysis, comprise the remainder of the stand of ponderosa pine growing in the 2 ha area. There is no evidence of logging or other major disturbance of this population in the recent past. After permanently tagging each tree, its diameter was measured, and its age was estimated from a count of annual rings from a wood core sample taken 30–50 cm above ground. Total fully developed mature cones (omitting obviously shrunk, aborted cones) were counted on each tree just prior to seed shedding. Mature needle samples taken from each tree were ground in liquid nitrogen, then mixed with a grinding solution to prevent binding of proteins by phenols, and centrifuged. The supernatants were subjected to horizontal starch gel electrophoresis. A peroxidase (PER) and two esterases (F.E., C.E.) each with three alleles were monitored for this study. Recipes of grinding buffers, electrophoretic methods and Mendelian inheritance patterns are published elsewhere (Mitton *et al.* 1977, 1979).

## 3. RESULTS AND DISCUSSION

In the population studied, cone production is positively correlated with both age and diameter of a given individual (Fig. 1), a result which agrees with findings published on ponderosa pine and other species of forest trees (Schubert, 1974; Stern & Roche, 1974; Dorman, 1976). To describe the relationship between cone production and both age and diameter of trees simultaneously, the data were treated by multiple regression analysis. Cone production is satisfactorily described by the following equation: cone production =  $-358.1 + 0.5(\text{age}) - 29.2(\text{diameter})$ . This equation describes a substantial proportion of the variance in cone production (35%,  $P < 0.001$ ), and shows that diameter is a very important determinant of cone production ( $P < 0.001$ ) whereas age is relatively less important ( $P > 0.60$ ).

When differential reproduction is considered in an ecological and evolutionary context, the most interesting members of a population are those whose reproductive output is significantly different from that expected for their age and/or size. To determine whether unusually high or low reproduction, as measured by cone production, has any genetic consequences, two groups of exceptional individuals are compared. One group of 50 individuals is composed of those trees which most exceed their predicted cone production, and a second group of 50 is composed of individuals that are farthest below their expected values (Table 1). Heterogeneity of allelic frequencies between these exceptional groups is tested

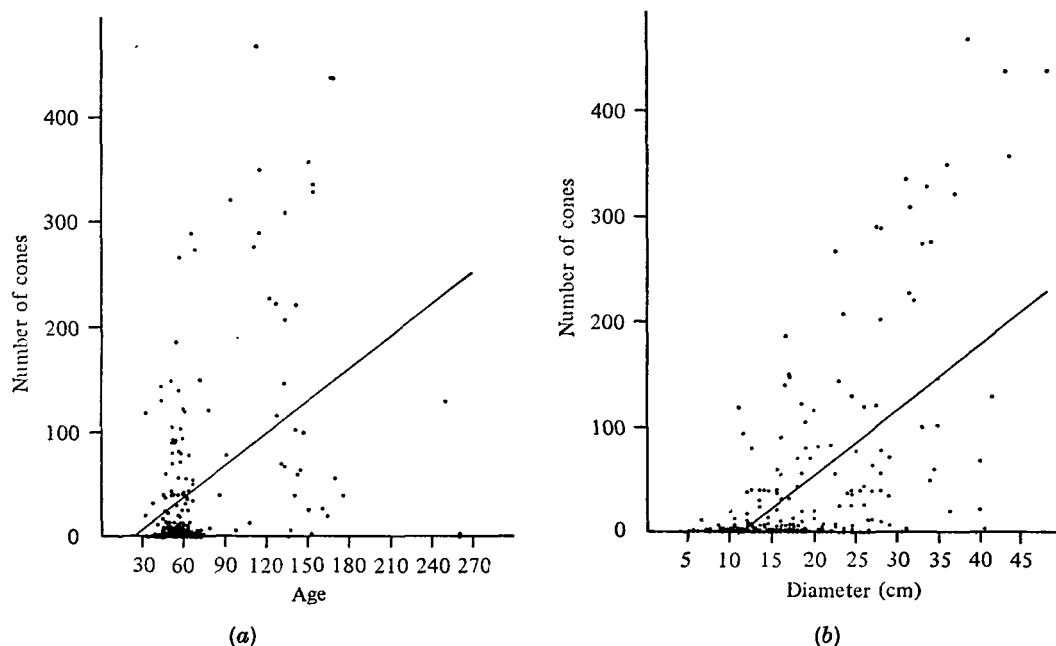


Fig. 1. Relationship between cone production and age and diameter of individual trees in ponderosa pine. (a) The relation between cone output and age, and the regression line is: Output =  $-123.7 + 5.1$  (age) with  $P < 0.001$ . (b) The relation between output and diameter, and the regression line is: Output =  $-354.1 + 3.1$  (diameter) with  $P < 0.001$ .

with a chi-square test that uses all three alleles at each locus, and has 2 degrees of freedom (Workman & Niswander, 1970).

As Table 1 shows there are significant differences between high and low cone-producers at all three loci. Heterogeneity is at the border-line of significance for the PER locus ( $P < 0.07$ ), while both the F.E. locus and the C.E. locus are heterogeneous across these groups ( $P < 0.05$ , for both loci). These groups differ in allelic frequencies by as much as 0.17 at the F.E. locus, and 0.15 at the C.E. locus, yet they come from the same hillside, and are defined only by their exceptional levels of cone production. Genotypic distributions also show differences between the two groups. The PER locus is significantly different in genotypic frequencies and the F.E. locus shows differences at the border-line of significance (Table 1). At both loci, the low fertility group approximates a Hardy-Weinberg equilibrium, but the high fertility group has an excess of heterozygotes. All other groups have a satisfactory fit to Hardy-Weinberg expectations.

Age, diameter and growth-rates (i.e. diameter/age) are also compared between the high cone-producers and low cone-producers. The high cone-producers as a group are slightly younger (64.8 years *v.* 67.4 years,  $P > 0.20$ ), but have significantly smaller diameters (14.8 cm *v.* 20.5 cm,  $P < 0.001$ ) and slower growth-rates (0.25 cm/yr *v.* 0.33 cm/yr,  $P < 0.001$ ). Thus, high cone-producers appear to produce large amounts of seeds at the expense of vegetative growth.

Table 1. *Comparisons in ponderosa pine between groups producing many or few cones*

At each locus, there are three alleles, numbered 1, 2 and 3. Genotype distributions are given first. Frequencies of the three alleles  $\pm$  standard errors are given next.  $\chi^2$  tests the fit of the genotypic distribution of a sample to Hardy-Weinberg expectations, and  $\chi^2$  tests heterogeneity of allelic frequencies at a given locus between high and low cone-producers.

Locus production	Genotypes									Allelic frequencies			$\chi^2$	Probability	$\chi^2$	Probability
	11	12	22	13	23	33	$F(1) \pm$ s.e.	$F(2) \pm$ s.e.	$F(3) \pm$ s.e.							
PER	0	7	21	0	22	0	0.070 $\pm$ 0.026	0.710 $\pm$ 0.045	0.220 $\pm$ 0.041	4.1	$P > 0.1$	5.5	$P < 0.07$			
	0	1	20	0	29	0	0.010 $\pm$ 0.010	0.700 $\pm$ 0.046	0.290 $\pm$ 0.045	8.8	$P < 0.05$	6.6	$P < 0.05$			
F.E.	1	15	28	3	3	0	0.200 $\pm$ 0.040	0.740 $\pm$ 0.044	0.060 $\pm$ 0.024	0.5	$P > 0.5$	5.6	$P < 0.07$			
	7	11	19	6	6	1	0.310 $\pm$ 0.046	0.570 $\pm$ 0.050	0.120 $\pm$ 0.035	1.8	$P > 0.5$	5.9	$P < 0.05$			
C.E.	20	5	0	22	0	3	0.670 $\pm$ 0.047	0.050 $\pm$ 0.022	0.280 $\pm$ 0.045	0.1	$P > 0.5$	0.1	$P > 0.5$			
	33	3	0	14	0	0	0.820 $\pm$ 0.038	0.030 $\pm$ 0.017	0.150 $\pm$ 0.035							

Data presented here suggest that differential reproduction as measured by cone production has a substantial genetic basis in ponderosa pine. Involvement in this phenomenon of all three loci monitored here coupled with the lack of significant association of age or detectable environmental features with expected cone production suggests that the genetic basis of cone production is fairly broad. Wolgast (1978) studying fertility differentials in the bear oak (*Quercus ilicifolia*) also concluded that genetic factors are of major importance in determining fertility in this tree.

The relative significance of this differential reproduction, observed during one year (1977), needs to be considered in the context of the long life-span of ponderosa pine. Successful reproduction in ponderosa pine is very infrequent in the southwestern U.S.A.: as noted above this particular population has only 22 individuals which were established in the past 33 years. We have been working in this area for several years and our observations suggest that 1977 was a year of heavy cone-production, an event which occurs every three to five years. This was followed by a wet spring in 1978, also a rare event. High moisture in spring and early summer is critical for seedling establishment in the southwestern U.S.A. and the combination of high seed output followed by high spring moisture may occur as rarely as every 60 years (Schubert, 1974). Therefore, differential seed production this particular year may have an important impact on the genetic composition of this population in the next generation.

In addition to the obvious evolutionary implications of these results, our observations are also germane to the design of field experiments. Samples that rely solely upon collection of seed will generate a biased estimate of genetic characteristics of the population studied whenever seed-producing individuals are genetically different from non-producing individuals.

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