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Reprinted for private circulation from THE AMERICAN NATURALIST,
Vol. 103, No. 932, July-August 1969, pp. 389-398

EMBRYONIC GENETIC LOAD IN COASTAL DOUGLAS-FIR, *PSEUDOTSUGA MENZIESII* VAR. *MENZIESII*

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Genetic load has been estimated for a number of outcrossing organisms, for example, *Drosophila* (Malogolowkin-Cohen et al. 1964), *Tribolium* (Levene et al. 1965), and man (Morton, Crow, and Muller 1956). However, little information about load of deleterious genes in higher plants has been published. The purpose of this article is to provide some data on plants by reporting an estimate of embryonic load in coastal Douglas-fir as determined from comparison of set of sound seed following self-pollination and following controlled crossing with unrelated pollen.

It was stated a decade ago (Dobzhansky 1957) that no estimates of frequency of deleterious and lethal genes were available for plants. This statement is still true in general, although some information does appear in the literature. For example, there are estimates or indications of load due to certain categories of genes in cultivated plants, especially genes causing chlorophyll deficiencies, as in maize (Crumpacker 1967) and pines (Eiche 1955; Snyder, Squillace, and Hamaker 1966). There are also data from some forest tree species, particularly coniferous species, which appear suitable for rough estimation of some part of the genetic load, although calculations of load have rarely been made (Fowler 1965*b* is an exception).

The present data from coastal Douglas-fir were originally collected for purposes other than calculation of genetic load. However, the "control" appears good enough and the number of trees sampled large enough that a well-based estimate of load is possible. Because of this and because load estimates on plants are rare, the results from coastal Douglas-fir are presented in detail here.

MATERIALS AND METHODS

Before outlining the experimental technique, I should say something about the ecology of Douglas-fir and its reproductive habits because both may affect the extent to which detrimental and lethal genes are accumulated.

Distribution

Douglas-fir grows throughout southwestern Canada, western United States, and in isolated localities in Mexico south to the vicinity of Mexico

City. The coastal type of Douglas-fir is recognized in the northwestern part of this range. In western Oregon, the location of this study, the species is ubiquitous except for a few arid valleys and where land has been cleared for agriculture (Sudworth 1908). Over a large portion of western Oregon, it is the major component of the forest.

Stand Structure and Outcrossing Potential

The amount of outcrossing that occurs prior to the establishment of a natural coastal Douglas-fir stand can only be very broadly estimated. However, there are some observations on pollen and seed dissemination that may help in making that estimate.

1. *Pollen dissemination.*—Individual trees bear both male and female reproductive structures. With rare exceptions, pollen shedding is coincident with receptivity of female strobili on the same tree (Orr-Ewing 1954). Pollen shedding occurs over a 7–15-day period on individual trees and over a 20–30-day period at a single location (Silen 1963). The contribution of self-fertilizations to total fertilizations is not known; however, it is known that air movement of pollen is extensive. In "one of the largest treeless areas" west of the Oregon-Washington Cascade Range, pollen capture was about 20% of what it was in the immediate vicinity of the forest (Silen 1962).

2. *Seed dissemination.*—Historically, coastal Douglas-fir has regenerated in stands opened up by fire (Isaac 1960) and in which scattered trees and patches of green timber were left after burns (Isaac and Meagher 1936). Under these conditions, seed dissemination is quite extensive; reasonably good reseeding occurs for an average distance of 600–700 ft and sparse reseeding up to 3,000 or more feet (Isaac 1930; Isaac and Meagher 1936).

Thus, it appears that *potentially* the effective population number of both female and male parents—but particularly male parents because of the greater dispersal of pollen—is high and that rate of cross-fertilization in coastal Douglas-fir is probably high. The evidence, to be presented later, of a large component of deleterious recessive genes is another argument against any substantial amount of inbreeding in this species.

Pollination and Fertilization

Pollination normally takes place from March through May, depending on latitude and altitude, and fertilization about 2 months later (Allen 1942). At time of fertilization, the female strobilus and female gametophyte (nutritive tissue for the embryo), if the latter is going to develop, are near full size. Both strobilus and gametophyte can develop fully without pollination or fertilization. Because nutritive tissue is near full size at time of fertilization, there is no requirement for double fertilization as in the case of angiosperms, and none has been observed (Allen 1946).

Following self-pollination, the progress of pollination, fertilization, and embryo development proceeds normally until 2 or 3 weeks after fertilization (Orr-Ewing 1956). At that time and for several more weeks, many embryos lose their structure, shrivel, and dry up. Gametophytic tissue then also dries up, and what is produced at the end of the season is a round, normal-appearing but empty seed.

Even though Douglas-fir is polyembryonic (more than one embryo commences development per ovule), the terminal embryo alone appears to determine the fate of the seed contents. Orr-Ewing (1956), who studied development of selfed embryos cytologically, says that ''collapse was general (i.e., included all material within the seedcoat) whenever the terminal embryo had collapsed and there was never any question of a second embryo taking its place.''

The normal Douglas-fir cone averages 40–50 ovuliferous scales, or positions for 80–100 ovules and potential round seeds. Of these, 10–70 usually fail to develop as the cone enlarges prior to fertilization. These latter remain flat against the scales and are incapable of being fertilized. This pattern occurs with or without pollination. These can be recognized as ''flat seeds'' at the time of seed extraction in the fall. All other seeds, usually 30–90 per cone, develop to full size regardless of whether or not they are sound. These are commonly referred to as ''round'' seeds.

Experimental Procedures

Thirty-five trees were cross- and self-pollinated at five locations along a west-east transect across the central Oregon Coast and Cascade Ranges. Each tree was pollinated in one or more of the years 1964, 1965, and 1966.

Fifty to 100 female strobili on each tree were isolated prior to bud opening and pollinated later with either self- or cross-pollen. Cross-pollen came from four to seven trees at least ¼ mile removed from the study trees. It was applied as a mix. Female strobili were uncovered for approximately 1–3 min while cross-pollen was being applied.

A different method of pollen application was originally used for selfing. In 1964 and 1965, trees were self-pollinated by enclosing male and female strobili in the same isolation bags, then shaking the bags when pollen was shedding and female strobili were receptive.

It was anticipated that the two methods of pollination, pouring on pollen for crossing and shaking pollen in bag for selfing, would be equivalent in their effectiveness. However, microscopic examination of the stigmatic area of crossed and selfed female strobili revealed a more thorough pollen cover when the pollen was poured on.

Consequently, in 1966, 12 trees were self-pollinated both by pouring pollen on the female strobili and by shaking pollen and female strobili in the same bag. Filled seed yields from the two methods of self-pollination were then related by use of ratios based on individual trees (Model IA, Snedecor 1956, p. 153). Ratios were determined for 11 of the 12 trees. (The twelfth tree was not used because its ratio differed greatly from any of the other 11, which indicated that a special technique problem was associated with that tree.)

Average ratio for the 11 trees was 1.29; seed yield after pollen was poured on was 1.29 times the seed yield after male and female strobili were bagged together. Self-fertilities of the 23 trees tested in previous years were then adjusted to make self- and cross-pollination comparable.

In the fall of the year, cones were collected, and 10 cones[1] from each treatment on each tree were taken apart and counts made of total round, round filled, and round empty seeds. Generally, from 300 to 700 seeds were classified for each treatment on each tree. However, some trees were tested in more than 1 year, and for these trees more seeds were classified.

Round seeds coming from the outcrosses included some empty seeds which were due to inadequate pollination and some perhaps due to unknown environmental causes. Round seeds coming from self-pollinations included empty seeds due to the same causes plus those resulting from increased homozygosity of lethal and detrimental genes. Therefore, the contribution of the latter could be estimated.

"Relative self-fertility," defined as the number of filled seeds per 100 round seeds following selfing as a proportion of the number of filled seeds per 100 round seeds following outcrossing, was the basis for estimation of embryonic lethal equivalents. ("Lethal equivalent" is used here in the sense of Morton et al. 1956 as "a group of mutant genes of such number that, if dispersed in different individuals, would cause on the average one death.")

There were no significant differences among the five localities in average relative self-fertilities, so all 35 trees are treated as if coming from a common population.

Determination of number of lethal equivalents followed Morton et al. (1956):

$$\begin{aligned} R &= \text{relative self-fertility} \\ &= \frac{\text{proportion good seeds at } F = 0.5}{\text{proportion good seeds at } F = 0.0} \\ &= \frac{e^{-A-0.5B}}{e^{-A}} \\ &= e^{-0.5B}, \end{aligned}$$

where F is the inbreeding coefficient, A is the expressed load in a randomly mating population, and B is hidden or concealed load that would be expressed fully only at $F = 1$.

Rearranged, $B = -2 \ln R$, the average number of lethal equivalents per gamete that makes up the "concealed" genetic load; and $2B = -4 \ln R$, the "hidden" genetic load on a zygote basis.

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[1] Six trees following selfing and seven following outcrossing yielded less than a 10-cone sample. Two had a three- and four-cone sample; the rest had five to nine cones.

RESULTS

Results of the pollinations are shown in table 1 and figure 1. They can be summarized as follows:

TABLE 1
Relative Self-Fertility and Calculated Number of Lethal Equivalents for Each of 35 Coastal Douglas-Fir Trees

Tree Number	Seed Yield[*]		Relative Self-Fertility[†]	Number Lethal Equivalents (Per Zygote)
	Cross (Filled seeds/round seeds)	Self		
EC-6[‡]	525/636	0/739[§]	.001	27.6
L-12[‡]	236/560	1/1500	.002	24.9
L-3	1201/1359	2/635	.003	23.2
SP-3	571/683	1/154	.007	19.8
L-4[‡]	366/712	3/646	.012	17.7
L-10[‡]	407/664	6/576	.022	15.3
EC-2	321/616	12/680	.034	13.5
L-32[‡]	120/194[‖]	10/546	.039	13.1
MP-5[‡]	1299/1660	5/236[‖]	.040	12.9
L-1	336/584[#]	26/733	.045	12.4
EC-4[‡]	112/545	15/692	.049	12.1
EC-5[‡]	184/271[#]	7/716	.061	11.2
C-26[‡]	430/655	19/610	.061	11.2
MP-7	275/337[#]	8/194[#]	.065	10.9
C-24	671/741	25/400	.068	10.8
SP-7[‡]	390/439	21/442	.069	10.7
L-5[‡]	269/1213	15/1190	.072	10.5
L-2	1042/1308	33/548	.075	10.4
C-28[‡]	229/525	9/350[#]	.076	10.3
SP-8[‡]	140/156[#]	17/266[#]	.091	9.6
C-25[‡]	314/649	24/671	.095	9.4
C-8	386/479	24/298	.099	9.3
SP-4[‡]	373/474[#]	21/313	.109	8.9
L-6[‡]	355/461	30/460	.109	8.9
MP-2[‡]	397/476	32/427	.116	8.6
MP-1[‡]	407/434	39/446	.120	8.5
L-11[‡]	481/596	47/516	.145	7.7
EC-3[‡]	403/493	48/511	.148	7.6
MP-6	373/533	72/544	.188	6.7
EC-1	175/234	75/466	.215	6.1
C-29	493/653	105/617	.225	6.0
SP-2	468/755	53/305	.281	5.1
L-9[‡]	484/542	109/513	.307	4.7
L-8[‡]	262/520[#]	74/437[#]	.432	3.4
SP-1	504/750	189/605	.464	3.1

[*] Results based on 10 or more dissected cones per treatment, except where indicated otherwise.

[†] Relative self-fertility is the quotient of "self-seed set" divided by "cross-seed set." Twenty-three trees marked with cross double dagger (‡) have also had relative self-fertilities adjusted to correct for a difference in cross- and self-pollination techniques. Pollination techniques and adjustment are described in text under "Experimental Procedures."

[‡] See footnote above.

[§] One or two sound seeds were obtained from other selfed cones, but not from the 10 that were dissected. A very low self-fertility was therefore assumed.

[‖] Results based on three to four dissected cones.

[#] Results based on five to nine dissected cones.

1. The median tree carried an estimated inbreeding genetic load of about 10 embryonic lethal equivalents per zygote.

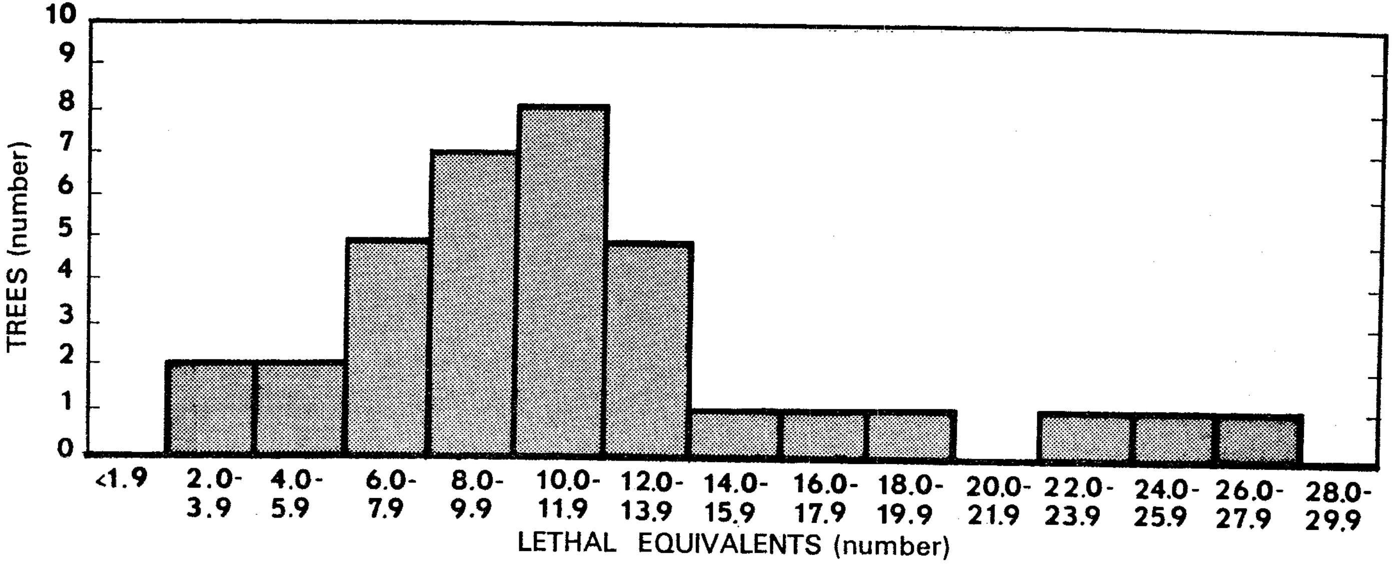

FIG. 1.—Frequency distribution of coastal Douglas-fir trees according to their embryonic genetic load

2. Range of estimated inbreeding loads among the trees was large, extending from 3.1 to 27.6 lethal equivalents.
3. Distribution of individuals according to their embryonic load tended to be skewed with the long tail toward the high number of lethal equivalents (fig. 1).
4. Correlation between outcross and self-seed sets was not significant. As is shown in table 1, both moderately self-fertile trees and those which were nearly self-sterile were capable of giving good seed yields when outcrossed.

DISCUSSION

A genetic load of 9–10 lethal equivalents per zygote (0.35–0.38 lethal equivalents per chromosome) is greater than has generally been reported for more thoroughly studied organisms. For example, 1–2 lethal equivalents per zygote have been reported for *Drosophila pseudoobscura* (Dobzhansky, Spassky, and Tidwell 1963), *D. willistoni* (Malogolowkin-Cohen et al. 1964), and *Tribolium* (Levene et al. 1965), and from 3–5 (Morton et al. 1956) to 1–10 per zygote (Freire-Maia, Guaraciaba, and Quelce-Salgado 1964) in man.

The high estimate for Douglas-fir may be partially due to the failure of deleterious genes to meet the assumption of independence in action. Results of Malogolowkin-Cohen et al. (1964) showed there may be synergistic interaction among deleterious genes even at low levels of inbreeding. In the present case, level of inbreeding is not low; seed yields are compared at F(inbreeding coefficient) $= 0$ and 0.50. On the other hand, load estimates for insects and man were generally based on $F < 0.25$. If synergism is important, then the inbreeding load reported for Douglas-fir will be somewhat overestimated relative to that given for other organisms.

In this comparison, the load for Douglas-fir is all expressed during embryo development, and that for other organisms is generally on an egg-to-fertile-adult basis. However, the difference between these bases may not be great because the major proportion of lethality following inbreeding in Douglas-fir occurs prior to germination and probably during early embryogenesis (Orr-Ewing 1957). (For example, the load for early post-germination period amounted to only 0.4 for 16 inbred families germinated from the above selfed seeds.) Some additional load will undoubtedly be expressed as the families mature; indeed, there is already evidence for some sterility load in older progenies of first-generation selfings (Orr-Ewing 1965). However, present conclusion must be that the majority of the genetic load in coastal Douglas-fir is expressed early. This is compatible with suggestion that, where a heavy genetic cost is levied against a long-lived organism, it is probably less serious to the species if a large portion of that cost can be borne in the seed stage (Haldane 1957).

The range of loads is approximately ninefold (table 1). However, this range, like the average, may be somewhat overestimated because of the above-mentioned synergism among deleterious genes. Overestimation may be particularly influential where the number of lethal equivalents is large, as is the case with the first few listed trees in table 1. Still, even with this possibility of overestimation, considerable variation among individual Douglas-firs is indicated.

Comparison with Other Conifers

Because the relationship between embryo deaths and empty seeds is not the same for all coniferous species, interspecific comparison has a weakness, namely, that it is based on relative self-seed sets and only approximately reflects comparative embryo deaths or comparative genetic loads. The reason for this is that conifers have the capacity to form more than one embryo within each ovule, that is, are polyembryonic, but not all species are polyembryonic to the same extent. For example, cytological study of Douglas-fir has indicated that, in this species, if the terminal embryo of any ovule aborts, as a result of homozygosis of deleterious recessive genes following inbreeding, the remaining contents of the ovule will also shrivel up and an empty seed will be formed (Orr-Ewing 1956). In this case, embryo death and empty seed will be equivalent and a relative self-seed set of 12% will indicate a relative self-embryo survival of 12%. But evidence indicates otherwise for *Pinus silvestris.* In that species, a second embryo may continue to develop even though one aborts within the ovule (Sarvas 1962). According to Sarvas (1962, p. 94–95), about 25% of the ovules in *P. silvestris* contain one developing embryo, and the remainder, two or more. If this species then has a relative self-seed set of about 35% (as calculated from Eklundh Ehrenberg and Simak 1956; and Plym Forshell 1953), it will have a relative self-embryo survival of only about 22%. Seed set and embryo survival will not be equivalent.

The behavior of other coniferous species with regard to effective polyembryony is not well described, although there is evidence that at least some other species of *Pinus* behave more like *P. silvestris* than like Douglas-fir (Sarvas 1962). In the comparisons that follow, it must be stressed that they are based on self-seed set or self-seed survival, not on embryo survival. The interspecific differences, however, in seed survival are large, and it may be reasonably inferred that variability in embryo survival and in embryonic genetic load is also large.

The author is aware of no conifers that are significantly less self-fertile than coastal Douglas-fir; but, as noted earlier, there are several whose self-seed set seems roughly comparable. These are *Picea pungens* (Cram 1964), *Picea abies* and *Larix decidua* (Dieckert 1964), and perhaps *Sequoia gigantea* (Yablokov 1960). *Pinus elliottii* appears to have only a slightly higher self-seed set (Kraus and Squillace 1964; Snyder 1968). There are also some species whose self-fertilities appear considerably greater than that of Douglas-fir. *Pinus banksiana* (Fowler 1965*b;* Rudolph 1967), *Pinus densiflora* (Katsuta 1966), *Pinus monticola* (Bingham and Squillace 1955), *Pinus silvestris* (Eklundh Ehrenberg and Simak 1956; Plym Forshell 1953), and *Pinus thunbergii* (Katsuta 1966) set on the average from 30% to 60% as many sound seeds following selfing as following outcrossing, and *Pinus resinosa* (Fowler 1965*a*) and *Picea omorika* (Langner 1959) seem to be fully self-fertile.

Thus, it can be seen that the situation described for coastal Douglas-fir does not apply to all coniferous species. Presumably, differences in breeding habits or genetic systems permit this wide variation in self-fertility and genetic load, but, for the most part, these differences are not at the present time well understood.

SUMMARY

Thirty-five coastal Douglas-fir trees were evaluated for embryonic genetic load from comparisons of sound seed set following self- and cross-pollinations, that is, from determinations of relative self-fertility. Estimates for the 35 trees ranged from about 3 to about 27 lethal equivalents per zygote active in the embryo stage, with the median tree carrying about 10 lethal equivalents per zygote. Relative self-fertility of Douglas-fir was compared with that reported for other coniferous species, and genetic load was compared with that reported for *Drosophila, Tribolium,* and man.

ACKNOWLEDGMENT

Acknowledgment is due to Drs. D. W. Crumpacker and B. Wallace for helpful suggestions and criticisms.

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