CHLOROPHYLL LETHAL IN NATURAL POPULATIONS OF THE ORCHARD GRASS (*DACTYLIS GLOMERATA* L.). A CASE OF BALANCED POLYMORPHISM IN PLANTS^{1,2}

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IN recent years extensive information has been gathered as to the occurrence and evolutionary significance of recessive lethal genes in natural panmictic populations (for details see reviews by DOBZHANSKY 1951, 1955, 1959). Work has concentrated almost exclusively on several *Drosophila* species, undoubtedly due to the fact that extraction techniques in this genetically well-studied genus are highly developed. Strikingly high frequencies of recessive lethals have been found to be a common feature in natural populations of various *Drosophila* species, and a model of balanced polymorphism has been suggested to account for these facts.

Compared to the genus *Drosophila*, information concerning the occurrence and distribution of recessive lethals in other organisms is rather meagre. An exception is the case of man; here high frequencies of the sickle cell factor in some human populations (ALLISON 1954, 1956) as well as those of several other deleterious recessive genes (NEEL 1956), have been explained on the basis of a model of balanced polymorphism.

In the present paper data are brought forward which indicate a case of balanced polymorphism, involving a recessive lethal in plants. It deals with the occurrence and distribution of a chlorophyll-deficiency factor in natural populations of diploid ($2n = 14$) orchard grass (*Dactylis glomerata*) in Israel. The study was initiated after it had been noted that certain seed samples collected for breeding purposes from individual wild *Dactylis* plants in the vicinity of Jerusalem, yielded a high proportion of white, chlorophyll-deficient seedlings (up to 12–15 percent). Since *Dactylis* is self-incompatible, such progeny should have originated from cross-pollination of a heterozygous parent by pollen carrying the mutant gene. On the assumption that an albino seedling is homozygous for a single recessive lethal locus, the appearance of up to 12–15 percent of albino seedlings among progeny of a given plant seemed to indicate that chlorophyll-deficiency genes were widely distributed in the local *Dactylis* populations.

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² The data presented are taken from the M.S. thesis submitted by the first author to the Dept. of Botany, The Hebrew University, Jerusalem.

MATERIAL

Both diploid ($2n = 14$) and tetraploid ($2n = 28$) forms of *Dactylis glomerata* L. occur in Israel (for details on their geographical distribution see NUR and ZOHARY 1959). The present work deals only with natural populations of the local diploid form: *D. glomerata* ssp. *judaica*. Tetraploids have not been investigated.

D. glomerata ssp. *judaica* occurs in Israel mainly in three areas: (a) the Judean Hills around Jerusalem, (b) Nazareth Hills, (c) the area of Safad, Upper Galilee. Here, occurring on several different soil types, this perennial grass constitutes a common component of the dwarf shrub and herbaceous plant communities (Mediterranean "batha" formations) which are a characteristic vegetational feature of these areas.

As to its breeding system, *Dactylis glomerata* ssp. *judaica* is a truly panmictic or cross-pollinated plant. Seed is produced sexually, and the plants are entirely self-incompatible. The latter fact has been verified by the present authors in numerous breeding experiments. No seed has ever been obtained upon the selfing of local *Dactylis* plants.

EXPERIMENTAL PROCEDURE AND RESULTS

The frequencies of chlorophyll-deficiency genes have been investigated in 14 samples taken from 13 chosen localities occupied by natural populations of *Dactylis glomerata* ssp. *judaica*. They are representatives of the main areas in which diploid orchard grass is known to occur in Israel and constitute a cross section of the various Israeli habitats occupied by this grass.

Sampling and seed harvesting were carried out at the end of the growing season, in July 1958 (8100-series) and in June 1959 (9100-series). Sampling procedures were as follows: At each station 100 plants were chosen for examination of their progeny, and seed from each plant was harvested separately in the field. Plants were sampled at each station along three or four transects which were 15–20 meters apart. Plants were sampled in pairs. The distance between pairs was 5–10 meters, within pairs 1–2 meters. The sampling of pairs of adjacent plants was employed to check on homogeneity of spread of heterozygotes in the examined areas. This was checked by means of a binomial test, the two events being identical pairs (i.e. both partners homozygous normal or both heterozygous) *versus* heterogeneous pairs (i.e. one partner homozygous normal, the second heterozygous). These tests indicated that the homozygous and heterozygous plants were distributed at random, with no local concentrations of one or the other genotype. There was no indication of uneven spread in the populations sampled.

Estimates of the frequencies of chlorophyll-deficiency genes in the various populations were obtained in two ways: (a) heterozygous plants were detected by means of progeny tests; (b) plants whose heterozygosity had been established were used as natural testers.

Detection of heterozygous plants by means of progeny tests: The seed sample collected from each respective plant was sown in the winter following harvesting

under greenhouse conditions in Jerusalem. The germinating seedlings were examined 3–4 weeks after sowing. The number of green (or normal) and albino (or chlorophyll-deficient) progeny obtained from each plant was recorded. A plant was assumed to be heterozygous for a chlorophyll-deficiency factor, when there was at least one albino seedling among its progeny.

The number of plants, in each locality, heterozygous for a chlorophyll-deficiency recessive, as detected by means of progeny tests is given in Table 1.

Naturally, the numbers of seeds that could be harvested from the individual parents varied greatly from plant to plant and from locality to locality. An effort was made to secure at least 50 seeds from each plant, but this was not possible in every case. Individuals which yielded less than ten seedlings were discarded from

TABLE 1

Detection of heterozygous plants: Results of progeny tests

Sample number	Locality and soil type	Total number of plants tested	Number of normal homozygous plants	Number of heterozygous plants	Percent of heterozygotes in sample	Estimated gametic frequency of albino gene (percent)
Jerusalem Hills:						
DC 8101:	Matzeleva Valley					
	terra-rossa	47	35	12	26	13
DC 8102:	Ora					
	rendzina	79	65	14	18	9
DC 8103:	Aminadav					
	rendzina	94	81	13	14	7
DC 8104:	Schneller Camp					
	terra-rossa	78	55	23	29	14.5
DC 8105:	University campus					
	terra-rossa	64	58	6	9	4.5
DC 8106:	Qiryat Anavim					
	terra-rossa	80	66	24	30	15
DC 9101:	Qiryat Anavim					
	terra-rossa	94	66	28	30	15
DC 9102:	Kastel					
	terra-rossa	100	72	28	28	14
Nazareth Hills:						
DC 8110:	Kefar Hahoresht					
	rendzina	82	79	3	4	2
DC 8111:	Nazareth					
	rendzina	95	90	5	5	2.5
Upper Galilee:						
DC 8107:	Ein Zeitim					
	rendzina	79	76	3	4	2
DC 8108:	Safad					
	rendzina	88	77	11	13	6.5
DC 8109:	Biriya					
	mixed soil	86	76	10	12	6
DC 8112:	Miar					
	rendzina	97	82	15	15	7.5

further considerations, whether they produced only green—or both normal and albino seedlings. This is the cause for the discrepancy between the 100 plants originally sampled at each locality and the results of the progeny tests presented in Table 1.

In some stations and particularly in the Jerusalem area, strikingly high percentages (26–30 percent) of plants yielded albino seedlings, thus indicating a large proportion of heterozygotes. It is probably safe to regard the plants which yielded albino progeny as heterozygous for a recessive chlorophyll-determining factor. But it is obviously impossible, solely by means of that method, to decide whether these plants are heterozygous for the same deficiency gene, or whether they represent several chlorophyll-determining factors at different loci. Yet the values obtained from utilization of heterozygotes as natural testers (see next section) strongly indicate that, at least within a respective locality, most of the plants which had yielded albino seedlings were actually heterozygous for the same recessive gene. The estimates of the gametic frequencies given in Table 1 were therefore calculated on the basis of such an assumption.

Heterozygotes as natural testers: Since plants of this taxon are self-incompatible and necessarily cross-pollinated, any individual whose heterozygosity had been established could be used as a natural tester for estimating the proportion of pollen, carrying the albino gene, produced in its vicinity. Any such pollen grain germinating on the stigma of a heterozygous plant has a 50 percent chance to produce a homozygous albino progeny. Assuming that there is no selective elimination (either in gametes or in zygotes), the percentage of albino seedlings in the progeny of a given heterozygote thus represents one half of the gametic frequency of the chlorophyll-deficient lethal and one fourth of the percentage of heterozygous plants within the sampled area.

In each locality, the data on progeny of plants which yielded albino seedlings (and were therefore assumed to be heterozygous) were also utilized for such an estimate of the gametic frequency of the chlorophyll-deficiency gene. To avoid complications, only heterozygotes which yielded 30 seedlings or more were included in these estimates. Table 2 summarizes the estimates of the gametic frequencies of the chlorophyll-lethal obtained upon utilization of the heterozygotes in each locality as natural testers.

In the majority of stations these estimates agree quite closely with the percentages of heterozygous plants as detected by means of the first method (compare Tables 1 and 2). The only major exception is sample DC 8105, where the values based on natural testers are exceptionally high and disagree entirely with the low number of detected heterozygotes. This general agreement between the two sets of data strongly suggests that at least within a given locality one is dealing mainly with a single lethal gene. Obviously, if several different factors for chlorophyll-deficiency had been present in a respective population, the frequency values as calculated from the testers (Table 2) would have been considerably lower than those based on detected heterozygotes (Table 1).

Ecological differences: The *Dactylis* populations examined were growing on two main types of soils. Some localities were characterized by typical mediter-

anean red-soil or "terra rossa". This soil type develops on a hard limestone and dolomite bed-rock. In other localities the soil was a light colored rendzina. This is a highly calcareous soil which is formed, in the mediterranean areas of Israel, on soft marls and chalks. Only in one case (DC 8109) the sample was collected in a place where the two soil types formed a mosaic. The soil type of each locality is noted in Table 1 and 2. Further ecological details on each sample may be obtained by any interested reader, by writing to the junior author.

As can be seen from the data set out in Tables 1 and 2, the frequency of the chlorophyll-blocking gene is much higher in *Dactylis* populations occupying terra rossa than in those occupying rendzina soil. In the Jerusalem area these

TABLE 2

Frequencies of the chlorophyll-deficiency gene as estimated by use of heterozygotes as natural testers

Sample number	Locality and soil type	Number of testers employed	Total number of green progeny	Total no. of chlorophyll deficient progeny	Total number of progeny	Estimated gametic frequency (percent)	Estimated frequency of heterozygotes (percent)
Judaean Hills:							
DC 8101: Matzeleva Valley							
	terra-rossa	7	375	25	400	12.5	25
DC 8102:	Ora rendzina	12	868	22	890	5	10
DC 8103:	Aminadav rendzina	13	1511	32	1543	4	8
DC 8104:	Schneller Camp terra-rossa	21	2069	145	2214	13	26
DC 8105:	University campus terra-rossa	5	318	43	361	24	48
DC 8106:	Qiryat Anavim terra-rossa	21	1448	95	1543	13	26
DC 9101:	Qiryat Anavim terra-rossa	20	2290	111	2401	9.5	19
DC 9102:	Kastel terra-rossa	18	3795	167	3962	8.5	17
Nazareth Hills:							
DC 8110:	Kefar Hahoresht rendzina	3	160	3	163	3.5	7
DC 8111:	Nazareth rendzina	5	432	5	437	2.5	5
Upper Galilee:							
DC 8107:	Ein Zeitim rendzina	3	420	7	427	3.5	7
DC 8108:	Safad rendzina	11	733	28	761	7.5	15
DC 8109:	Biriya mixed soil	10	718	16	734	4.5	9
DC 8112:	Miar rendzina	15	2995	70	3065	4.5	9

differences are highly indicative, since stations on different soil types are geographically in close proximity (distances of 3–4 km), and the *Dactylis* cover of this area is more or less continuous.

DISCUSSION

The most plausible explanation of the results obtained in the progeny tests is the occurrence of a recessive, chlorophyll-blocking allele in relatively high proportions in some natural populations of the diploid orchard grass in Israel. That one is dealing mainly with a single locus in each respective population is indicated by the agreement between the number of detected heterozygotes (Table 1) and the estimates based on natural testers (Table 2). Although the most likely situation is that of a simple Mendelian trait, this has yet to be verified. The final evaluation of the results obtained can be made only after genetic analysis has been carried out in at least some of the albino-producing plants, and after it has been confirmed that they are actually simple heterozygotes, and that one is dealing with the same locus in the various samples.

As to the detection technique employed, it is realized that some heterozygotes could have been missed, and erroneously considered as normal homozygotes, because a limited and variable number of progeny (ten to several hundreds) were raised from each tested plant. This is particularly true in populations where the frequency of the albino allele was found to be low. However, such errors would not change the general picture obtained.

The high proportions of the recessive lethal gene are best explained on the assumption of a higher selective value of heterozygous plants as compared to that of homozygous normal individuals. An equilibrium of adaptive polymorphism for a recessive lethal is proposed to account for the results obtained. This assumption of higher selective values of heterozygotes is further supported by the differences noted between populations occupying terra rossa and these growing on light colored rendzina soil, which indicate different selective values in different ecological niches.

Finally, attention should be drawn to the possibility that a chlorophyll-blocking class of mutations might be of general evolutionary significance in plants. The studies of WILLIAMS and BROWN (1956) on the cherry demonstrate that selection for quantitative economic traits has favored heterozygosity for chlorophyll-blocking recessives. Apparent vegetative vigor was found by GUSTAFSSON (1946) and GUSTAFSSON, NYBOM and VON WETTSTEIN (1950) in barley heterozygotes for induced, recessive chlorophyll mutations. The diploidization of the *W*s locus in *Nicotiana tabacum* reported by CLAUSEN and CAMERON (1950) might be regarded as being of similar significance. The data presented in this paper indicate that such lethals can maintain themselves in a panmictic population. Adaptive polymorphism involving chlorophyll-deficiency factors is probably not restricted to the orchard grass alone. It may be of a more general occurrence in cross-pollinated plant species.

SUMMARY

Natural populations of diploid ($2n = 14$) orchard grass (*Dactylis glomerata*) native in Israel were examined as to the frequency of recessive chlorophyll-deficiency lethal in them. Frequencies up to 30 percent of heterozygous plants (yielding albino progeny) were found in some localities. It was also noted that populations growing on terra-rossa soils show higher proportions of the lethal gene than populations occupying rendzina soils.

The most likely explanations for the results obtained is that in certain habitats, heterozygotes for a chlorophyll-blocking lethal have a higher selective value than normal homozygotes. Apparently a condition of balanced polymorphism with regard to this locus is present in the populations of the orchard grass tested.

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LITERATURE CITED

- ALLISON, A. B., 1954 Notes on sickle cell polymorphism. *Ann. Human Genet.* **19**: 39-49.
1956 The sickle cell and haemoglobin C genes in some African populations. *Ann. Human Genet.* **21**: 67-89.
- CLAUSEN, R. E., and D. R. CAMERON, 1950 Inheritance in *Nicotiana tabacum*. XXIII. Duplicate factors for chlorophyll production. *Genetics* **35**: 4-10.
- DOBZHANSKY, TH., 1951 *Genetics and the Origin of Species*. 3rd Ed. Columbia Univ. Press. New York.
1955 A review of some fundamental concepts and problems of population genetics. Cold Spring Harbor Symposia Quant. Biol. **22**: 385-393.
1959 Variation and evolution. *Proc. Am. Phil. Soc.* **103**: 252-263.
- GUSTAFSSON, A., 1946 The effect of heterozygosity on viability and vigour. *Hereditas* **32**: 263-286.
- GUSTAFSSON, A., N. NYBOM, and U. WETTSTEIN, 1950 Chlorophyll factors and heterosis in barley. *Hereditas* **36**: 383-392.
- NEEL, J. V., 1956 The genetics of human haemoglobin differences: problems and perspectives. *Ann. Human Genet.* **21**: 1-30.
- NUR, U., and D. ZOHARY, 1959 Distribution patterns of diploid and tetraploid forms of *Dactylis glomerata* L. in Israel. *Bull. Research Council Israel* **7D**: 13-22.
- WILLIAMS, W., and A. G. BROWN, 1956 Genetic response to selection in cultivated plants: Gene frequencies in *Prunus avium*. *Heredity* **10**: 237-245.