with metamorphism regardless of origin. Moreover, a significant degree of graphitization occurs by natural process when the layers attain a size of about 26 A, as compared to 100 A or more by the heat treatment of amorphous carbons.

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Effects of Supernumerary Chromosomes on Production of Pigment in Haplopappus gracilis

Abstract. One of the two types of supernumerary chromosomes found in *Haplopappus gracilis* effects pigment production in the achene walls. When one, two, and four supernumerary chromosomes were added to the basic complement, a corresponding increase in the amount of one type of pigment was found to occur. No overlapping of the effects on pigment production was observed among different numbers of supernumeraries or between the supernumeraries and normal chromosome complement.

Supernumerary chromosomes have been reported for many plant and animal species (*1*). They have been found to be devoid of genes in the usual sense, but in certain species a reduction in vigor, fertility, and sexual maturity can be attributed to their presence (*2*). Some of these effects are correlated with increased numbers of supernumeraries (*3*). However, attempts to relate a definite phenotypic effect with a certain supernumerary chromosome have been unsuccessful thus far. A possible exception may obtain in *Plantago*, in which one extra chromosome has been found to be associated with male sterility (*4*).

In a previous report of the supernumerary chromosomes in *Haplopappus gracilis* (Nutt.) Gray (*5*), it was pointed out that plants containing the larger type of supernumeraries could be distinguished by certain morphological characteristics of the leaves and stems. In addition, the achene coats of plants with supernumerary chromosomes were found to be a dark red color while the normal chromosome type was usually brown or, rarely, reddish at maturity.

The purpose of this preliminary study was to determine whether the larger type of supernumerary chromosomes exhibited an additive effect on pigment production in the achene coat as they were increased in number in the plant.

Using seed from reciprocal crosses of plants with $2n = 4 + 2$ and $2n = 4 + 3$, we grew a number of progeny in the greenhouse with normal diploids ($2n = 4$) having brown or reddish achenes. The chromosome numbers of all the plants were determined from somatic cells of immature heads. Several plants with chromosome numbers of $2n = 4 + 1$ and $2n = 4 + 2$ were obtained, but only two plants with $2n = 4 + 3$ and one with $2n = 4 + 4$ were grown to maturity. The mature achenes were harvested, dated, and stored at room temperature until used.

The pigments were extracted from ten weighed achenes in 3 ml of cold HCl in 90 percent ethyl alcohol, in a Tenbroeck all-glass tissue grinder, and the nonsoluble cell debris was removed by centrifugation. The solution containing the pigment from the brown achenes was yellow, while that from the reddish achenes and the red supernumerary fruits was varying shades of pink. Spectrophotometric analyses were carried out in a Beckman model DU spectrophotometer. Weight differences of the achenes were corrected after analysis.

The yellow pigment solutions, after dilution, yielded absorbance maxima at two points, 335–340 mμ and 270–275 mμ. The latter peak sometimes appeared only as a small shoulder in the absorbancy curve. The pink pigment solutions (undiluted) gave absorbance maxima at three points, one at 535–540 mμ, and the other two at or near the same wavelengths as the yellow after equivalent dilution. It thus appears that two and possibly three different pigments are present and that the yellow pigment occurs in the reddish diploid type as well as in plants having the supernumerary chromosomes.

In Fig. 1, the absorbance maxima of the two higher wavelengths of one sample have been plotted for each of the normal and aneuploid types. As the figure indicates, there is little or none of the pink pigment present in the brown achenes with a normal chromosome number. However, the data show a greater amount of the pink pigment in the achenes with supernumeraries than is found in the reddish type with the normal chromosome complement. In addition, there is an increase in the amount of pigment when one, two, and four supernumeraries are added to the normal complement. The plants with three supernumeraries produced less pigment than those with one and two, but it is important to note that this amount was somewhat greater than it is for the reddish diploid type and that it did not overlap with any of the other supernumeraries.

The yellow pigment occurred in greatest quantities in the brown achenes. A lesser amount was found in the reddish type, and the quantity varied in the achenes of plants with different numbers of supernumerary chromosomes. Whether there is a relationship between the general decrease in the amount of yellow pigment and the increase in red pigment in plants with up to two supernumeraries remains to be determined. A direct relationship might be expected if the two pigments were dependent upon a common precursor. An explanation is wanting also for the peculiar effect of three supernumeraries on the production of the red pigment. Nevertheless, the importance of the data presented here lies in the fact that each supernumerary chromosome exerts a definite effect upon the production of both yellow and pink pigments, and that no overlapping of the effects was found.

Genetic analysis, now in progress, should yield information on the mode of inheritance of pigment production.

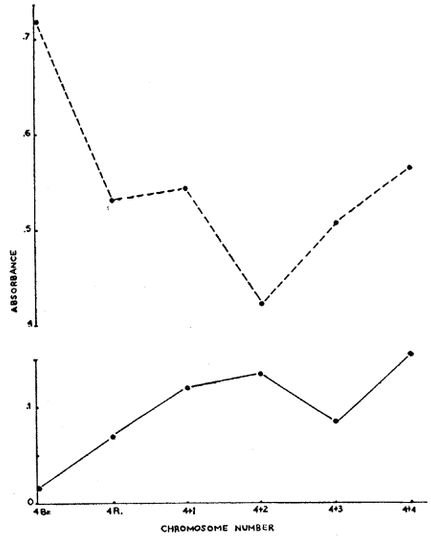

Fig. 1. Upper curve (dashed lines) shows absorbance maxima at 335–340 mμ of brown (4B) and reddish (4R) diploid achenes, and aneuploid types with one to four supernumerary chromosomes. The lower curve shows the absorbance maxima at 535–540 mμ for the same chromosome types.

Although the data presented here are admittedly meager, it would seem that the supernumerary chromosomes carry genes for pigment production which are similar to or the same as those on the normal chromosomes (*6*).

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Estimate of the Human Load of Mutations from Heterogeneous Consanguineous Samples

Abstract. A formula is presented for the calculation of the mean number of lethal and abnormal equivalents per person. It has been applied to Brazilian, French, and Japanese data.

A number of the methods for the estimation of the mutational load in man are based on procedures in which only one class of consanguineous marriages is used (see *1*). For samples containing marriages with different degrees of consanguinity, a more general formula may be developed as follows:

The probability that a zygote from a consanguineous marriage will be homozygous for any one of the alleles present at a specific locus in the common ancestors is given by the coefficient of inbreeding. Suppose that each one of the common ancestors, considered here to be average individuals, is a carrier of a rare deleterious recessive mutation. The probability that the zygote will be homozygous for derivatives of any one of the deleterious genes is given by $f/2$. If we suppose now that the average individual carries not one, as postulated above, but D deleterious recessive mutations, the probability of homozygosity for any one of the D deleterious genes turns out to be $Df/2$. This value can be obtained by analyzing the frequency, $\bar{x}$, of deleterious recessive homozygotes in the offspring of consanguineous marriages. Thus,

$$\bar{x} = Df/2 \quad \text{and} \quad D = 2\bar{x}/f \quad (1)$$

By a different reasoning Penrose (*2*) and Slatis *et al.* (*3*) came to the conclusion that in the special case of full first-cousin marriages ($f = 1/16$), $D = 32\bar{x}$.

Now, given the fraction of abortions, miscarriages, stillbirths, mortality from birth to the mean marriage age, and anomalies, due to homozygosity for recessive genes, we could obtain the mean number, per person, of lethal equivalents acting in the different stages of development, as well as the mean number of abnormal equivalents. The summation of all these values would give us the total mean number of deleterious equivalents per person:

$$D = \sum_k \frac{2\bar{x}}{f} \quad (2)$$

In samples containing not one but different types of consanguineous marriages, the frequency of homozygotes due to inbreeding is given by the mean coefficient of inbreeding:

$$\bar{f} = \sum_{j \neq 0} \frac{f_j n_j}{N} \quad (3)$$

where f_j is the jth coefficient of inbreeding, n_j is the number of pregnancies (for data on abortions and miscarriages) or children born (for stillbirths) or children born alive (for mortality from birth to the mean marriage age, and anomalies) associated with f_j, and N is $\sum_j n_j$. A rigorous analysis would score a monozygous twin pregnancy as one event and a dizygous twin pregnancy as two, but the use of any pregnancy—single or twin—as one event will introduce only a trivial error. Substituting for f in formula (2) the value $\bar{f}$, we obtain:

$$D = \sum_k \frac{2N\bar{x}}{\sum_{j \neq 0} f_j n_j} \quad (4)$$

In cases of mortality it is impossible to differentiate deaths caused by recessive genes from those caused by other factors. In such cases, as well as for anomalies in general, the frequency $\bar{x}$ of recessive homozygotes cannot be detected. It is possible, however, to obtain a rough estimate of $\bar{x}$ by subtracting the rates of mortality or anomalies in a suitable control sample (S_c) from those rates in the consanguineous (inbred) sample (S_i). Substituting (S_i-S_c) for $\bar{x}$ in formula (4), we get

$$D = \sum_k \frac{2N\,(S_i - S_c)}{\sum_{j \neq 0} f_j n_j} \quad (5)$$

that is, an estimate of the mean number of deleterious equivalents per individual. This formula does not correct for the error introduced into the data by those deaths where the individual was simultaneously homozygote for two or more lethals, or semilethals. Since the probability of this event is rather small, the error introduced would appear negligible.

When S_i is lower than S_c, D will take a negative value. This will not have genetic meaning with respect to deleterious equivalents and may be interpreted as an accident of sampling. If D is based on large samples, a negative value may be interpreted as indicating a very low mean number of deleterious equivalents per person.

Formula (5) has been applied to data on abortions plus miscarriages, stillbirths, and mortality from birth to the mean marriage age, from some Brazilian populations (*4*). The mean number of lethal equivalents per individual in the whole sample has been found to be 1.55. A large difference was found, however, between the two ethnic groups involved in the analysis; the mean number was −0.37 for Caucasians (almost all of Portuguese ancestry) and 9.12 for Negroes (*5*). The method of Morton, Crow, and Muller (*6*) has also been applied to these Caucasian and Negro data and lead to estimates close to those obtained according to formula (5): −0.24 for Caucasians and 10.46 for Negroes (*1*).

Formula (5) has been applied to Schull's (*7*) and Sutter and Tabah's (*8*) data and gave results similar to those obtained by the method of Morton *et al.* (*6*; *9*).

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