distinct variation in inbred color and, through alteration of photosynthetic capacities, for the significant differences in grain production in the single crosses with the tester stock. Work is now in progress to determine chromosome loci of the three genes described and to discover and isolate other genes of the pigment control series. These data as well as a more complete publication concerning the genetic, economic and evolutionary importance of this genic series will be prepared in the near future.

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*REPRODUCTION AND DISTRIBUTION OF THE CYTOPLASMIC FACTOR FOR MALE STERILITY IN MAIZE**

BY WARREN H. GABELMAN

DEPARTMENT OF GENETICS, CONNECTICUT AGRICULTURAL EXPERIMENT STATION, NEW HAVEN, CONNECTICUT

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Recent work of Preer,[1, 2] Sonneborn[3, 4] and L'Héritier,[5, 6] has emphasized and helped clarify the rôle of the cytoplasm in the determination and inheritance of characters. The present paper deals with the inheritance of a cytoplasmic factor in maize in such a way as to determine particle number, rate of reproduction of the particle and the distribution of the particles

within the plant. This is the same cytoplasmic factor previously studied by Rhoades.[7] Similar cytoplasmic factors causing male sterility have been found in onions by Jones and Clarke[8] and in sugar beets by Owen.[9] In both of these latter cases, an interaction between the cytoplasmic factor and chromogenes was reported.

Rhoades[7] showed that microsporogenesis was normal and that the abortion occurred during the development of the microspore into a mature male gametophyte. If the plant was partially fertile, certain of these microspores would continue development normally. When this partially fertile pollen was used in cross-pollinations there was no evidence of a transfer of the male sterile factor. This fact suggested the possibility of the presence of one or more cytoplasmic factors in a microspore destined to abort and the complete absence of the factor in those microspores which would develop into normal pollen grains.

Partially fertile lines were selected for a detailed study of variability in pollen production among florets of a given plant. Such a study would rule out the possibilities of genic and physiological differences which might normally arise in a comparison between plants. The use of partially fertile plants makes possible the detection of small differences not measurable in completely sterile lines.

The amount of viable pollen was determined by smearing the pollen from the anthers of a single floret in a drop of aqueous iodine-potassium iodide solution. Those pollen grains which were plump and full of starch were considered viable. If there were any shrunken, starch-deficient or otherwise aborted areas in the pollen grain, it was considered to be non-viable. Cross-pollinations using the partially fertile types as pollen parents were successful in direct proportion to the amount of viable pollen shed per plant as determined by this method.

Five tassels from partially fertile plants were chosen at random. The per cent of viable pollen was determined by observing 150–200 pollen grains per floret, using a Breed micrometer to limit the amount of the field counted. Counts were made at a magnification of 150 ×. Five such counts at random usually included 150–200 pollen grains. When such data from any individual tassel were subjected to a $2 \times N$ chi square test, the variation among the florets exceeded that expected by chance ($P = <0.001$). Frequency distributions were made of the percentages of good pollen produced in these florets. Such frequency distributions were bimodal or polymodal with only one approaching unimodality. The modes of these distributions were at 36 and 60 per cent viable pollen. Figure 1 presents these data combined to show these peaks more clearly.

Each of the five plants used in figure 1 when studied by itself was free of genic and physiological differences so that such polymodal differences must have been due to the cytoplasmic factor itself. Since the modes

from these five plants were at the same points it would indicate that the same system was involved in each case.

An explanation for such a distribution may be formulated in the following manner. Assume the presence of k discrete cytoplasmic particles in the microsporocytes which give rise to aborted pollen grains. Since functional pollen produced by a partially fertile plant does not transfer the cytoplasmic factor it can be assumed to be free of the male sterile particle. It follows that the aborted pollen grains contain one or more of these particles. The particle number must remain constant through successive cell divisions. If, at mitosis, the particles were distributed to daughter cells by chance alone, cells should arise devoid of the particle. The descendents of such a cell should all be devoid of particles and a

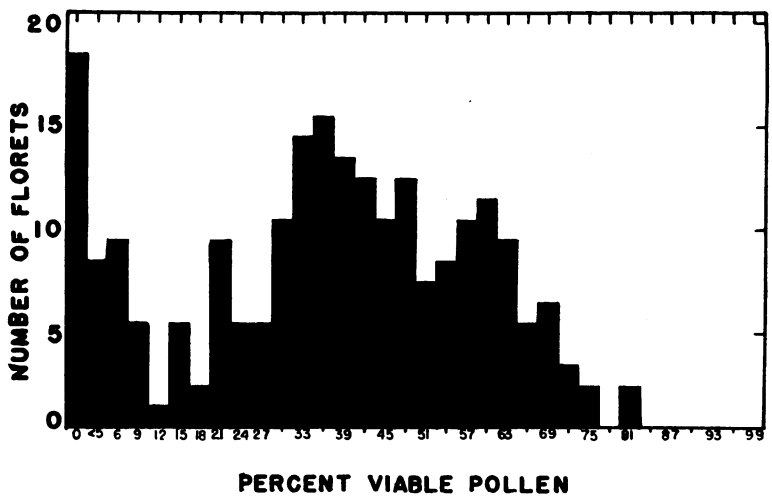

FIGURE 1

Frequency distribution of number of florets plotted against percentage of viable pollen, grouped in intervals of 3 per cent, accumulated from 5 plants.

pollen fertile chimera should result. No such chimeras have been found in any of the plants tested. From inspection of the variability in figure 1, it is evident that at some late cell division, such as during meiosis, the control which previously had insured equal division must be impaired. If this distribution at meiosis were random, a Poisson series would be expected.

The Poisson series is the basic discontinuous random distribution. It may occur when a variable is not able to take all possible values, but is confined to a particular series of values, such as whole numbers. If a variate can take the values 0, 1, 2, . . ., X, . . ., and the relative frequencies with which the values occur are given by the series

$$e^{-m}\left(1, m, \frac{m^2}{2!}, \ldots, \frac{m^x}{x!}, \ldots\right)$$

then the number is distributed in the Poisson series.[10] The mean is the only unknown parameter of the Poisson series. If m is the mean number of particles per pollen grain, the proportion of samples containing none is e^{-m}. The pollen grains, free of the particle, would be viable. If each peak (Fig. 1) represented the mean of a component Poisson series, the average number of particles per pollen grain would be $^1/_2$ and 1 for 60 and 36 per cent viable pollen, respectively.

If the number of particles per cell were constant throughout the plant and only two types of pollen could be identified, pollen free of the particles and pollen containing one or more particles, it should be possible to plot the percentage of viable pollen in successive samples as a unimodal frequency distribution. Figure 1 is polymodal although only two modes predominate.

Two possibilities may explain such a divergence from the Poisson. Occasionally particle division and cell division might not coincide during mitoses, leading to an increase or decrease in the number of particles per daughter cell. Alternately, the distribution of particles to daughter cells may not always be exact.

Either of these possibilities could lead to a heterogeneous population of particles within the cells of a single plant. Random samples drawn from such a population would take the form of a negative binomial distribution.[11] In experimental sampling the negative binomial arises from a single extension of the conditions underlying the Poisson series. The Poisson series arises when equal samples are taken from perfectly homogeneous material. It is completely determined by the average number of particles, m per sample. If unequal samples were taken, or, if the material was not perfectly homogeneous, the value of m would vary from sample to sample.

If an atypical division happens only occasionally, a stage should be reached as meiosis is approached where a significant discrepancy in particle number and consequently in the percentage of viable pollen would be expected in some cases and not in others. This can be tested by comparing the percentages of good pollen in the anthers of the two florets which comprise the spikelet. Although still many cell divisions from meiosis, the anthers represent a much later common derivation than can be claimed for florets on different spikelets. In consequence, a $2 \times N$ chi square test should show agreement with the binomial in some cases and not in others. Comparison of the viable pollen in the anthers of the five spikelets studied showed probabilities of 0.78, 0.22, <0.01, <0.001 and <0.001. This shows that a binomial distribution is fit occasionally as meiosis is approached which is in agreement with the proposed hypothesis.

If the male sterile factor is distributed at random during microsporogenesis, it is of fundamental interest to see what type of distribution is had during megasporogenesis. Is this random distribution a function of meiosis?

This problem was tested by studying six florets (at random) from each of 78 plants from a three-way cross. These data are shown graphically in figure 2.

In making such counts the accuracy is very limited below 10 per cent and above 90 per cent. Actual counts were only made on those florets about 5 per cent or more viable. It should be noted that three definite peaks can be found at 15, 36 and 57 per cent. This is in agreement with

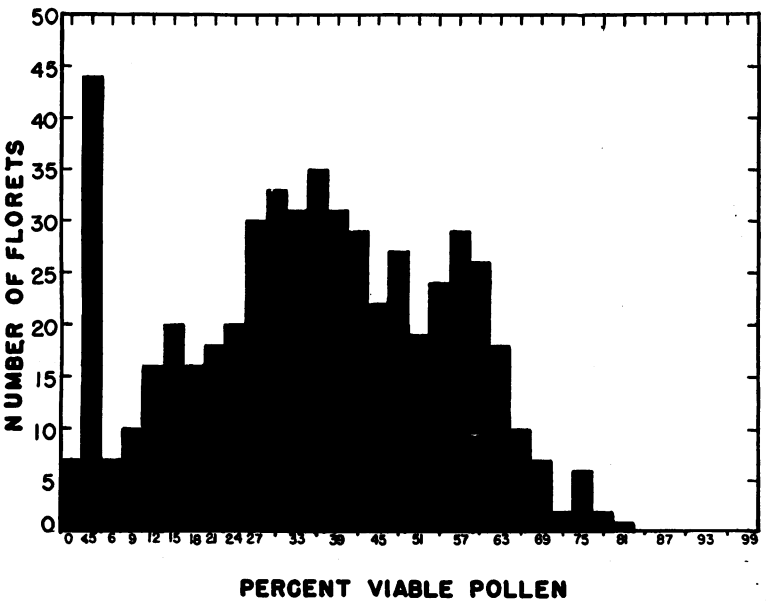

FIGURE 2

Frequency distribution of number of florets plotted against percentage of viable pollen, grouped in intervals of 3 per cent, accumulated after determining per cent of good pollen in 6 florets at random on each of 78 plants.

mean particle numbers of 2, 1 and $^1/_2$, respectively. It should also be noted that the variability is greater in figure 2 than in figure 1, which indicates that the random distribution probably took place at megasporogenesis of the parent plant as well as microsporogenesis of the progeny and is a function of meiosis.

The female plant in the three-way cross used in this study produced 49 per cent viable pollen in 1947. This corresponds to 0.714 particle per pollen grain. The median of the florets (Fig. 2) was 0.741 particle. In

a second study the female had 0.891 particle per pollen grain (1947) and the progeny 0.967 particle per pollen grain (1948). These data suggest that there was no appreciable increase or decrease in the number of particles per cell during the life cycle of these two families.

One of the basic premises of the Poisson series is that the variate must be confined to a particular series of values, such as whole numbers. If the number of particles per cell must be one or more (as shown previously by the absence of pollen fertile chimeras), a mean number of $^1/_2$ needs to be justified.

The assumption that the particle number is relatively constant at mitosis seems to be valid. It has also been assumed that at some stage, presumably at meiosis, the forces in control during mitosis are absent and distribution of the particle becomes random. Since the division of particles presumably paralleled that of the chromosomes during mitosis a similar parallelism during meiosis would suppress particle division at the same time and lead to the random distribution of undivided particles. If the cytoplasmic particles were to divide only once during meiosis as do the chromosomes, a mean number of 1 per somatic cell will be reduced to $^1/_2$ per microspore. Similarly, peaks arose at $^1/_2$ and 1 particle per microspore. Never was there any variance from this distribution. Therefore, the numerical difference between these peaks ($^1/_2$) should be the equivalent of a whole particle per somatic cell. Similarly modes were not found between 0 and $^1/_2$ particle per cell.

Discussion.—The significance of plasmagenes has been the subject of much speculation. Sonneborn[12] has recently reviewed the numerous views presented by many workers. From the evidence presented here it is possible to characterize partially the cytoplasmic factor causing male sterility in corn.

The male sterile factor is particulate. If it were not a whole unit or particle its distribution would not follow a negative binomial made up by a series of Poisson distributions.

The reproduction and distribution of this particle are quite similar to the reproduction and distribution of chromosomes. The similarity in reproduction holds both at meiosis and mitosis. The similarity of distribution holds quite well at mitosis but is completely random at meiosis. It must, therefore, have many characteristics in common with the chromosome. It might also be suggested that this is a virus. All attempts to transfer it artificially have failed. It is doubtful that a virus would be so dependent on chromosome division for its reproduction and distribution.

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*THE EFFECT OF OXYGEN ON THE FREQUENCY OF X-RAY INDUCED CHROMOSOMAL REARRANGEMENTS IN TRADESCANTIA MICROSPORES**

BY NORMAN H. GILES, JR., AND HERBERT PARKES RILEY†

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY

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The recent studies of Thoday and Read,[1] using root tips of *Vicia faba*, indicate that the availability of oxygen to cells is a very important factor in radiosensitivity. Under anaerobic conditions, sensitivity to x-rays is markedly reduced, as measured both by inhibition of growth and by frequency of anaphase figures showing chromosome aberrations. The results of Hayden and Smith[2] on x-radiation of barley seeds in a vacuum also suggest that the absence of oxygen results in reduced radiosensitivity. In view of these results it seemed of interest to test the effect of oxygen on the frequency of x-ray induced chromosomal rearrangements in Tradescantia, especially since there is already available a wealth of information on radiation effects in this organism as a result of the pioneer investigations of Sax[3] which, together with those of other investigators, have been summarized by Lea[4] and by Catcheside.[5]

Materials and Methods.—Tradescantia paludosa Anderson and Woodson, clone 5 of Sax, was used in all experiments. Observations were made at the first postmeiotic mitosis in the microspore by means of acetocarmine smear preparations at the four- to five-day interval following treatment, at which time only chromosome-break types are present. The principal aberration types analyzed at this period are interchanges (dicentrics and centric rings). In addition, interstitial deletions (intercalary deletions or isodiametric fragments) are numerous. Acentric rings and terminal deletions were also recorded but, because of their comparative rarity, were not used in later calculations.