# Linkage Relationship between *Ga*-Factors and Genetic Markers of Maize Chromosome 9

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**Summary.** Self-pollination of plants derived from normal seeds of *Ga Wx/ga wx* selfed ears has given, over a period of ten years, 54 ears with about 25% *wx* kernels plus 600 ears showing either no *wx* kernels or only a very few (about 4% *wx*). From these figures a crossover rate of about 8.7% for the distance between *Wx* and the *Ga* factor, found by the senior author, can be calculated.

A similar procedure for the repulsion phase *Ga wx/gaWx* (originally and independently found by SCHWARTZ and SALAMINI) leads to an estimate of about 13% as a c.o. distance between *Wx* and *ga* (555 ears with a large excess of *wx*, 152 with 25% *wx* kernels, 105 ears with no *wx*, and 5 ears with a great deficiency of *wx*). The same factor was located between *Wx* and *Bz*, at about 2/3 of the *Wx-Bz* distance from *Wx*.

Selfed ears of plants *Ga sh C/ga Sh c* give an excess of *sh* (about 36.3%), and a deficiency of *c* (15.3%). If *ga* gametes are assumed to be non functional (as indicated by other results), these data suggest that *ga* is about 27 c.o. units from *sh*, and 31 from *c*. A distance of the same order of magnitude from *sh* is determined from ears of selfed plants of genotype *Ga Sh/ga sh* which show 12% *sh* kernels.

An additional chromosome 9 marker, closely linked to *ga*, is an albino seedling factor (*w*). The data indicate that the albino factor is closer to *Ga* than *wx*; they also permit to estimate the c.o. value between *wx* and *w* (about 2±0.2%).

If *ga*-gametes do not function, the percentage of *w* seedlings (about 2 %) doubled (4%) provides an estimate of the c.o. value between *w* and *ga*.

The distance *wx*—*w* was also obtained as variable between 0 and 7 c.o. units.

The linkage map of the genes in the short arm of chromosome 9, on the basis of the available data, is tentatively as follows:

$C$ 3,5 $sh2$ $bz$ 20 $Ga_{8-2 \text{ and } 8-3}$ $O$(?) $Ga_{8-1}$ 2.5 $w_{11(?)}$ 3.5 $wx$.

Genetics has developed and has given origin to phases characterized by interests in various phenomena bearing on heredity, and, as have most sciences, by certain methodological approaches. An analysis of MENDELS data (FISHER, 1965) has shown that most of them "have been falsified so as to agree closely with" his expectation. If this is true, preliminary results lead MENDEL to establish an hypothesis to which successive adjusted data were associated, perhaps by too zealous an assistant "who knew too well what was expected". In other words, typical Mendelian ratios appeared repeatedly to conform even too closely to the expectation already in MENDEL's hands, more than 100 years ago.

With the rediscovery of MENDEL's laws, at the beginning of this century, genetics grew as a stolon of biological sciences which seemed to many to be destined merely to grind out once again endless 3:1 ratios, more or less precisely. It appeared so much so that, as S. WRIGHT mentions (1959), more than one geneticist decided to go into a different field, because genetics was considered a "cow that had been milked".

Actually, although the endless Mendelian ratios were continuously and correctly accumulated, they soon changed from being a geneticist goal to a genetical tool. At the same time genetics became inextricably linked with several biological sciences — from cytology to systematics, from ecology to biochemistry — as well as with exact sciences — from statistics to physics, from chemistry to radiation research, and so the real core of modern biology. However, this evolution of genetics, whilst making greater use of typically Mendelian ratios and Mendelising traits, has witnessed the arousal of other quantitative aspects.

The genetics of characters presenting continuous variation apart, non-Mendelian ratios soon made their appearance in the history of genetics. (Of course, the consequences of gene interaction and linkage do not alter the basic Mendelian nature of the factors involved in such phenomena.)

It is rather interesting, in fact, to mention that just after the rediscovery of MENDEL's laws, CORRENS (1902) observed significant deviations in the segregation of *su* (sugary) kernels of some maize plants heterozygous for alleles of this locus. He interpreted such deviations, as a consequence of a differential transmission of the gametes carrying the two allelomorphs. This was a partially correct hypothesis: in fact, EMERSON (1925) suggested that the excess or the deficiency of *su* kernels on ears of *Su su* self-pollinated plants was due to the presence in the chromosome carrying *Su* or *su* of a gametophyte factor responsible, respectively, for a normal or a deficient fertilization.

MANGELSDORF and JONES (1926) determined the linkage relationships of *su* and the gametophyte factor ($Ga_1$). Their work was rather an important change from the point of view of Mendelism: although unnoticed under this respect, it certainly contradicted the idea that genetics was the "cow already milked", at least because it was no longer an accumulation of 3:1 ratios. The common classification involves the distinction of individuals on the basis of their morphophysiological traits. MANGELSDORF and JONES, to measure the linkage intensity between the *su* and $Ga_1$ loci, classified the ears (in which *Su*, *su* segregation was observable) according to their Mendelian or "non-Mendelian" ratios.

The same criterion has been adopted in later studies of linkage relationships between gametophyte factors and known chromosome markers, especially for $Ga_2$ on chromosome 2 (BRIEGER, 1937), and for $Ga_8$ on chromosome 9 (BIANCHI and PARLAVECCHIO, 1966).

This work deals with the location of gametophyte factors of chromosome 9 in respect of well-known genetic markers of its short arm. Although the consistency of the results, throughout the complex series of data, is not complete, they offer convincing evidence of the alteration of Mendelian ratios brought about by some Mendelian factors in Mendelising traits, as well as strong suggestions, albeit not always conclusive, of the linkage relationship of the gametophyte factors and other genetic markers of chromosome 9.

The suggestion has been made of using gametophyte factors in maize breeding especially in view of hybrid seed production (PERRY 1945; BIANCHI 1961). The linkage relationship between genetic markers and gametophyte factors are of critical importance for the introgression of the latter in the inbred lines.

## Material and methods

The stocks carrying gametophyte factors in chromosome 9 which were used in this work are of three independent origins. A first case of individuals $Ga_8\ wx/ga_8\ Wx$ was found by SCHWARTZ (1952). In a mais-teosinte derivative, BIANCHI (1957) detected ears of constitution *Ga Wx/ga wx*. Some test-cross data indicated that the two factors were identical or allelic (BIANCHI, 1958); other results suggested that this might not be the case (BIANCHI and PARLAVECCHIO, 1966). Consequently, the SCHWARTZ factor is indicated by $Ga_{8-1}$, the other one by $Ga_{8-2}$. A third case of a gametophyte factor linked with *wx* was found by F. SALAMINI, who turned over to the authors ears of the genotype $Ga_{8-3}\ wx/ga_{8-3}\ Wx$.

In this paper are reported data, obtained from ears, produced by self-pollinating plants, grown from *Wx* seeds from ears of the three stocks mentioned above, together with some results of crossing the SCHWARTZ stock with strains possessing other markers of chromosome 9, namely *C*, *sh*, *bz*, at 30, 27 and 25 map units, respectively, from *wx* and a *w* (white) seedling trait placed at few units from *wx*.

A few special progeny tests and some test-crosses are also mentioned in connection with the testing and the control of the precise nature of genotypes which are not sufficiently self-explanatory on the basis of their behaviour.

The linkage relationship between the markers and especially the cross-over values, from data derived from genotypes involving gamete discrimination, may be ascertained in different ways, as has been discussed and as will be shown in the presentation of the various results.

The study of the pollen behaviour in germination by direct observation of fixed material has been carried out according to the methods described by BIANCHI and PARLAVECCHIO (1967).

## Results

Behaviour of *ga* and *Ga* pollen grains. Plants heterozygous for gametophyte factors produce *ga*- and *Ga*-pollen grains at the same rate. However, the functioning of the *ga*-pollen rests chiefly upon the genotype of the silk on which such pollen germinates, as well as upon the presence of *Ga*-pollen. The germination of *ga*-pollen is so slow on *Ga*-stigma that, in competition with *Ga*-grain, the efficiency of the former to effectuate fertilization was estimated by EMERSON (1934) at 4% of the normal $Ga_1$.

The behaviour of the *Ga*, *ga* pair of chromosome 9 has been studied by crossing plants $Ga_{8-2}\ Wx/ga_{8-2}\ wx$ with *Ga Ga wx wx* plants. As reported by BIANCHI and PARLAVECCHIO (1966), out of a total of 672 kernels only 32 were *wx*. The remaining 640, heterozygous for *Wx*, *wx*, are expected to be *Ga Wx/Ga wx* or *ga Wx/Ga wx*: the latter class is the result of crossing-over between *Ga* and *Wx*, and concomitant functioning of the *ga* grain. Actually, self-pollination of 25 plants derived from the *Wx*-kernels resulted in ears all with 25% of *wx*-kernels. Although the sample is small, the absence of individuals with excess of *wx*-kernels (which should be produced by plants *ga Wx/Ga wx*) is not at variance with the assumption that the *ga*-grain does not normally function on *Ga*-silk, in competition with *Ga*-grains. Another indication of the rare or nul functioning of the *ga*-pollen is obtained by an appropriate testing of plants producing 25% of *wx*-kernels in their self-pollinated ears. Obviously such plants must be heterozygous *Wx*, *wx*, but homozygous either for *ga* or for *Ga*. Similar genotypes are found, in a given frequency, in the progeny of the genotypes *Ga Wx/ga wx* and *Ga wx/ga Wx*, following self-pollination, depending on the cross-over rate between *Ga* and *Wx* loci. A dozen ears, exhibiting a regular Mendelian ratio, have been tested and have proved to be *Ga*-, which again points to the poor functioning of *ga*-gametes.

Linkage intensity between gametophyte factors and *wx*. In stocks possessing gametophyte factors closely linked to genetic markers, self-pollination of the plants produce mainly ears with significant excess or deficiency, respectively, of the markers according to the existence of a repulsion or of a coupling phase. In both cases an estimate of the linkage between the gametophyte factor and the genetic marker can be obtained by proper progeny analysis.

Ears, from self-pollinated plants, showing about 4% of *wx*-kernels (BIANCHI and PARLAVECCHIO, 1966) are the consequence of the plant genotype $Ga_{8-2}\ Wx/ga_{8-2}\ wx$. Planting of *Wx*-kernels from such ears, followed by self-fertilization of the ensuing plants, has produced the data condensed in Table 1.

In the column "Other ear types" are included not only those exhibiting a low percentage (on average, 4%) of *wx*-kernels, but also ears showing only *Wx*-kernels. This has been done because it has been firstly inferred, (BIANCHI and PARLAVECCHIO, 1966) and then shown, that part of the ears uniformly non-*wx* were actually *Wx Ga/ga wx*, *wx*-kernels being

Table 1. *Frequency of ear types produced by self-pollination of plants grown from Wx- seeds of* $Ga_{8-2}\ Wx/ga_{8-2}\ wx$ *ears.*

| Years | No. of ears segregating 25% of *wx*-kernels | No. of other ear types | Total No. of ears |
|---|---|---|---|
| 1956 | 2 | 53 | 55 |
| 1957 | 6 | 63 | 69 |
| 1958 | 0 | 4 | 4 |
| 1959 | 4 | 53 | 57 |
| 1960 | 3 | 37 | 40 |
| 1961 | 26 | 276 | 302 |
| 1962 | 2 | 18 | 20 |
| 1963 | 5 | 15 | 20 |
| 1964 | 2 | 41 | 43 |
| 1965 | 1 | 13 | 14 |
| 1966 | 3 | 27 | 30 |
| '56/'66 | 54 | 600 | 654 |

absent by chance.* (The probability of realising this, with ears with less than 100 kernels, when the expected *wx*-kernels rate is few units percent, is obviously high.)

From the data reported in Table 1 it is possible to calculate the crossing-over rate for the region *Ga-Wx*. Assuming non-functioning of *ga*-gametes, the average percent value is estimated at 8.67 ± 3.88.** In fact, under these circumstances, the possible zygotic combination produced by self-fertilization of the plants *Ga Wx/ga wx* are as follows ($p$ stands for crossing-over rate):

| Male Gametes | Female Gametes<br>*Ga Wx*<br>$\frac{1}{2}(1-p)$ | <br>*Ga wx*<br>$\frac{1}{2}p$ | <br>*ga Wx*<br>$\frac{1}{2}p$ | <br>*ga wx*<br>$\frac{1}{2}(1-p)$ |
|---|---|---|---|---|
| *Ga Wx* | $\frac{Ga\ Wx}{Ga\ Wx}$ | $\frac{Ga\ wx}{Ga\ Wx}$ | $\frac{ga\ Wx}{Ga\ Wx}$ | $\frac{ga\ wx}{Ga\ Wx}$ |
| $1-p$ | $\frac{1}{2}(1-p)^2$ | $\frac{1}{2}p(1-p)$ | $\frac{1}{2}p\,(1-p)$ | $\frac{1}{2}(1-p)^2$ |
| *Ga wx* | $\frac{Ga\ Wx}{Ga\ wx}$ | $\frac{Ga\ wx}{Ga\ wx}$ | $\frac{ga\ Wx}{Ga\ wx}$ | $\frac{ga\ wx}{Ga\ wx}$ |
| $p$ | $\frac{1}{2}p\,(1-p)$ | $\frac{1}{2}p^2$ | $\frac{1}{2}p^2$ | $\frac{1}{2}p\,(1-p)$ |

Disregarding the combinations homozygous for *wx* (from which it is impossible to learn the gametophyte constitution), the other formulae may be grouped in two classes, according to the presence of a regular Mendelian ratio (around 25% of *wx*-kernels), or to the absence of *wx*-kernels plus their presence in a percent of few units. The total rate (subtracting the *wx wx*-classes $\frac{1}{2}p^2 + \frac{1}{2}p\,(1-p)$) is $1 - \frac{1}{2}p$. The 25% class is $(1-p)p$: the actual corresponding figure is indicated by $x$. The remaining ears are $y$. Consequently the following equation may be written:

$$\frac{x}{x+y} = \frac{(1-p)\,p}{1-\frac{1}{2}p}$$

which gives:

$$(x+y)\,p^2 - p\left(\frac{3}{2}x + y\right) + x = 0$$

and, with the actual figures:

$$654\,p^2 - 681\,p + 54 = 0\,.$$

The acceptable solution of this equation is 0.0867. A very close estimate can also be gained, under the same assumption made of no function of *ga*-gametes on *Ga*-silk, from the percentage of *wx*-kernels in self-fertilized $Ga_{8-2}$ *Wx*/$ga_{8-2}$ *wx* plants (see Table 2). Obviously this percent value must be doubled to obtain the cross-over rate: (3.82 × 2 = 7.64). However, the percentage of the *wx*-kernels is calculated disregarding the undetermined fraction of ears $Ga_{8-2}$ *Wx*/$ga_{8-2}$ *wx* exhibiting no *wx*-kernels. Consequently this is an over-estimate. In spite of this the correspondence with the previous value is satisfactory, which implies that the number of such ears should not be high and that the previous estimate was not seriously biased when calculated according to the assumptions made.

The linkage relationships between $Ga_{8-1}$ and *wx*, as well as between $Ga_{8-3}$ (SALAMINI material) and *wx*, are treated together because of the strict resemblance in their behaviour, although the data are reported separately (see Table 3). By using the same method described above, it is possible to estimate the linkage intensity between these *Ga*-factors and *wx*. In this case three separate estimates can be obtained:

a) making use of the class segregating 25% of *wx*-kernels, which leads to a value of 11.79 ± 3.44%.

The calculations in algebraic form are as follows:

$$\frac{x}{x+y} = \frac{p\,(1-p)}{\frac{1}{2}+\frac{1}{2}p}$$

$$(x+y)\,p^2 - p\left(\frac{1}{2}x + y\right) + \frac{1}{2}x = 0$$

$$817\,p^2 - 741\,p + 76 = 0\,.$$

b) using the class comprehending ears with no *wx*-kernels or very few, which yields the estimate 13.35 ± 3.42.

Table 2. *Percentage of wx-kernels in ears produced by self-fertilization of plants* $Ga_{8-2}$ *Wx*/$ga_{8-2}$ *wx.*

| Year | No. of *wx*-kernels | Total No. of kernels | % of *wx*-kernels |
|---|---|---|---|
| 1959 | 123 | 4109 | 3.00 |
| 1960 | 170 | 3778 | 4.50 |
| 1961 | 715 | 20765 | 3.44 |
| 1962 | 57 | 1740 | 3.27 |
| 1963 | 45 | 1021 | 4.41 |
| 1964 | 167 | 2470 | 6.76 |
| 1965 | 25 | 366 | 6.83 |
| 1966 | 53 | 1170 | 4.53 |
| '59/'66 | 1355 | 35419 | 3.82 |

Table 3. *Frequency of ear types produced by self-fertilization of plants grown from Wx-seeds of Ga wx/ga Wx ears. The material marked "Sal" refers to Dr.* SALAMINI'*s case* (=$Ga_{8-3}$); *all other material is derived from* SCHWARTZ *stocks.*

| Year | No. of ears segregating *wx*-kernels with a ratio of about: — 3:1 | 6:4 | No. of ears with no *wx*-kernels | No. of ears with very few *wx*-kernels | Totals |
|---|---|---|---|---|---|
| 1959 | 0 | 14 | 2 | 0 | 16 |
| 1960 | 5 | 29 | 1 | 0 | 35 |
| 1961 | 58 | 215 | 40 | 4 | 317 |
| 1964 | 17 | 47 | 10 | 0 | 74 |
| 1964 (Sal) | 18 | 92 | 9 | 0 | 119 |
| 1965 | 23 | 66 | 22 | 0 | 111 |
| 1965 (Sal) | 3 | 16 | 4 | 0 | 23 |
| 1966 | 26 | 50 | 15 | 1 | 92 |
| 1966 (Sal) | 2 | 26 | 2 | 0 | 30 |
| 59/'66 | 152 | 555 | 105 | 5 | 817 |

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* Pollen samples of ears of this material, exhibiting all starchy kernels, have been stained with iodine solution and 3 out of a total of 12 so analysed turned out to possess 1/2 pollen staining blue (*Wx*-type) and 1/2 staining brown (*wx*-type).

** The standard error has been calculated by the formula $\frac{(2+p^2)\,(1-p^2)}{2\,n\,(1+p^2)}$ (HOGBEN 1946).

Table 4. *Percentage of wx-kernels in ears produced by self-fertilization of plants Ga wx/ga Wx.*

| Year | No. of *wx*-kernels | Total No. of kernels | % of *wx*-kernels |
|---|---|---|---|
| 1959 | 1024 | 2354 | 43.50 |
| 1960 | 3432 | 8204 | 41.83 |
| 1961 | 19795 | 49257 | 40.19 |
| 1964 (Sal) | 8755 | 20095 | 43.57 |
| 1964 | 3360 | 8294 | 40.51 |
| 1965 (Sal) | 1105 | 2523 | 43.80 |
| 1965 | 5329 | 13240 | 40.25 |
| 1966 (Sal) | 2432 | 5728 | 42.45 |
| 1966 | 2674 | 6982 | 38.30 |
|  | 47906 | 116677 | 41.06 |

Table 5. *Segregation of wx-kernels in ears derived from self-fertilization of plants Wx wx homozygous for gametophytic factors.*

| Year | No. of ears | No. of *wx*-kernels | Total No. of kernels | % of *wx*-kernels |
|---|---|---|---|---|
| 1960 | 5 | 414 | 1746 | 23.7 |
| 1961 | 54 | 3134 | 13832 | 22.7 |
| 1964 | 35 | 1742 | 7275 | 23.9 |
| 1965 | 24 | 1186 | 5417 | 21.9 |
| '60/65 | 118 | 6476 | 28270 | 22.9 |

The calculations in algebraic form are as follows:

$$\frac{x}{x+y} = \frac{\frac{1}{2}p^2 + \frac{1}{2}p}{\frac{1}{2} + \frac{1}{2}p}$$

$$\frac{x+y}{2}p^2 + \frac{y}{2}p - \frac{1}{2}x = 0$$

$$408.5\,p^2 + 353.5\,p - 55 = 0\,.$$

c) a third percentage value of 12.56 ± 3.43 is derived from the concomitant use of various classes. The calculations in algebraic form are as follows:

$$\begin{array}{r} 817.0\,p^2 - 741.0\,p + 76 = 0 \\ \underline{-\,408.5\,p^2 - 353.5\,p + 55 = 0} \\ 408.5\,p^2 - 1094.5\,p + 131 = 0 \end{array}$$

Although the last estimate may appear more reliable from the statistical point of view, actually the second one rests upon an unquestionable classification, for the practical impossibility of misclassifying the ears when the b) criterion is considered. Such an estimate is also reasonably close to the estimate that can be derived from Table 4 which reports the percentage of the *wx*-kernels in the ears produced by the self-fertilization of *Ga wx/ga Wx* plants. This percentage is about 41%. Assuming again the non-functioning of the *ga*-gametes the difference between the expected value in case of complete linkage and this percent is half of the crossing-over rate. Extensive data show that in our genetic background the *wx*-kernel percentage (as often is the case on the basis of maize geneticists' experience), in the absence of gametophyte differences, is 23% in self-pollinated heterozygotes (see Table 5). Consequently, the adequate figure to be used from which to subtract 41 is 48, which gives a cross-over value of 2 × 7 = 14 in good agreement with the estimates reported above, and especially with the value obtained on the basis of the rate of ears without or with very few *xw*-kernels.

Linkage relationships between gametophyte factors and chromosome 9 markers. The data so far analysed demonstrate clearly the existence of at least a gametophyte factor closely linked to *wx*-locus on chromosome 9. The next logical step in the work is the localization of the factor on the right or the left of the *wx*-locus in the linkage map. Obviously this can be done by studying the deviation of the Mendelian ratios in genotypes involving, besides the gametophyte pair, other genetic markers whose position in respect to *wx*-locus is known, and it has been done for the Schwartz and Salamini material.

Table 6 shows segregation of *c*- and *sh*-kernels on ears produced by self-pollinating plants of constitution *Ga sh C/ga Sh c*. The excess of the *sh*-class, as well as the deficiency of the *c*-kernels, are obvious. They are of a similar order of magnitude, as expected on the basis of the strict linkage of the factors *c* and *sh*. In fact, the standard cross-over value for the region *c-sh* is 3%. If the average value of about 15.3 for *c*-kernels is doubled, the distance between the gametophyte factor and *c*-locus turns out to be approximately 30.6. The deviation from 50 of the average value of 36.3 for *sh* being 13.7, the distance in percent units of crossing-over for the region of *sh-ga* is then 27.4. Table 7 reports data from plants *Ga Sh/ga sh*: the 12% value of the *sh*-kernels suggests a 24 estimate for the distance *ga-sh*. All these data are consistent with the hypothesis of a gametophyte factor linked to the two chromosome 9 markers, with

Table 6. *Segregation of c- and sh-kernels in ears derived from self-fertilization of plants Ga sh C/ga Sh c.*

| Ear Identification | No. of *c*-kernels | No. of *sh*-kernels | Total No. of kernels | % of *c*-kernels | % of *sh*-kernels |
|---|---|---|---|---|---|
| 66-504-3 | 30 | 70 | 181 | 16.57 | 38.67 |
| -4 | 54 | 118 | 350 | 15.43 | 33.71 |
| -5 | 40 | 90 | 253 | 15.81 | 35.57 |
| -9 | 54 | 147 | 363 | 14.88 | 40.50 |
| -505-2 | 23 | 70 | 187 | 12.30 | 37.40 |
| -4 | 26 | 94 | 235 | 11.10 | 40.00 |
| -8 | 28 | 67 | 187 | 14.97 | 35.82 |
| -17 sib | 49 | 94 | 319 | 15.36 | 29.46 |
| -506-7 | 43 | 91 | 243 | 17.69 | 37.45 |
| -10 | 32 | 91 | 219 | 14.61 | 41.55 |
| -507-1 | 39 | 85 | 242 | 16.11 | 35.12 |
| -508-1 | 31 | 98 | 276 | 11.23 | 35.51 |
| -4 | 43 | 117 | 309 | 13.92 | 37.86 |
| -9 | 54 | 111 | 315 | 17.14 | 35.24 |
| -510-4 | 49 | 128 | 340 | 14.41 | 37.65 |
| -5 sib | 37 | 93 | 255 | 14.51 | 36.47 |
| -6 | 40 | 80 | 200 | 20.00 | 40.00 |
| -7 | 42 | 72 | 216 | 19.44 | 33.33 |
| -9 | 10 | 25 | 82 | 12.20 | 30.49 |
| -10 | 22 | 34 | 116 | 18.97 | 29.31 |
| Totals | 746 | 1775 | 4888 | 15.26 | 36.31 |

Table 7. *Segregation of sh-kernels in ears derived from self-fertilization of plants Ga Sh/ga sh.*

| Ear identification | No. of *sh*-kernels | Total No. of kernels | % of *sh*-kernels |
|---|---|---|---|
| 66-509-4 | 40 | 244 | 16.39 |
| -5 | 24 | 279 | 8.60 |
| -6 | 23 | 265 | 8.68 |
| -7 | 24 | 182 | 13.19 |
| -8 | 42 | 361 | 11.63 |
| -9 | 30 | 280 | 10.71 |
| -12 | 29 | 155 | 18.71 |
| Totals | 212 | 1766 | 12.01 |

a tentative linkage map of the following types: *c* 3.5 *sh* 26 *ga*.

Also available were some ears segregating for a seedling trait, of the lethal albino type, closely linked with *wx*,* as is evident from the following data obtained in $F_2$ segregation:

| Linkage phase | *Wx W* | *Wx w* | *wx W* | *wx w* | c.o. ± st. err. |
|---|---|---|---|---|---|
| R | 2021 | 977 | 887 | 4 | 7 ± 1 |
| C | 311 | 3 | 0 | 110 | < 1 |

Crossing of the coupling phase plants with the gametophyte stocks of Dr. Salamini and subsequent self-pollination lead to genotypes producing distorted segregation for both *w* and *wx*, as is shown in the following prospect:

*Wx*-kernels

| Normal seedling | *w*-seedling |
|---|---|
| 2500 | 3 |

*wx*-kernels

| Normal seedling | *w*-seedling |
|---|---|
| 28 | 47 |

The data indicate that the albino factor is closer to *Ga* than *wx*. The percentage of the *wx*-kernels is about 3%, which suggests a value of about 6 c.o. units for the distance *Ga-Wx*; the corresponding value of the region *Ga-W* is approximately 2.5. The same data permit to estimate the c.o. value between *wx* and *w*: 2 ± 0.2% in good agreement with that obtainable from the pooling of the data reported above. Altogether the data from the segregation of alleles of the loci *Wx*, *W*, and *Ga* suggest the following tentative linkage map:

*Ga* 2.5 *W* 3.5 *Wx*

## Discussion

The data reported in this paper demonstrate clearly the occurrence of systematic deviations from the Mendelian expected ratios of segregation of genetic traits when the same chromosome pair carries alleles controlling such traits and the functioning of the gametophytes. So the experiments with chromosome 9 markers are confirmatory, under this respect, of what had already been illustrated with genetic markers of chromosomes 4 and 5, as reported in the literature mentioned in the introduction. But the experiments described were also programmed for locating the gametophyte factor (s) in the linkage map. From this point of view, however, the results require some discussion.

As reported in the introduction three independent examples of gametophyte factors have been found in chromosome 9. Allelism tests for similar genes are impracticable. However, crossing of the $Ga_{8-1}$ stock with the $Ga_{8-2}$ stock suggested that the two factors were actually identical or allelic (Bianchi, 1958): but this rested upon the fact that a discrimination in the pollen transmission was inferred by the distorted Mendelian ratio of the *Wx*, *wx* segregation. Although the interpretation of the results in such experiments was made according to the simplest hypothesis, they can be equally well explained admitting the coexistence of a second gametophyte locus on the same chromosome pair. The differential behaviour in germination of the pollen in the two stocks carrying $Ga_{8-1}$ and $Ga_{8-2}$ factors favours the latter possibility, although the difference can be reasonably explained also admitting an influence of the different genetic background or the existence of different alleles at the same locus (Bianchi and Parlavecchio, 1967). Under these circumstances, the study of linkage relationships between such gametophyte factors and genetic markers in chromosome 9 could have produced more critical information. Actually this has been obtained, but not conclusively, from all standpoints because of a partial inconsistency of some data.

The standard linkage map of the short arm of chromosome 9 is known as follows:

*C* 3 *sh* 2 *bz* 28 *wx* .

On the basis of the results described in this paper, the various linkage relationships that can be derived may be summarized in the following prospect: —

| Genetic region | Test | c.o. units ± st. err. | approx. pooled value of c.o. |
|---|---|---|---|
| $Ga_{8-2}$-*Wx* | progeny | 8.7 ± 3.9 | 8 |
| | pollen transmission | 7.6 | |
| $Ga_{8-1}$-*wx* | progeny a) | 11.8 ± 3.4 | 11 |
| | progeny b) | 13.3 ± 3.4 | |
| | progeny a + b) | 12.6 ± 3.4 | |
| | pollen transmission | 14 | |
| | pollen transmission | 6 | |
| $Ga_{8-2}$-*sh* | pollen transmission | 24 | 26 |
| $Ga_{8-2}$-*Sh* | pollen transmission | 27 | |
| $Ga_{8-2}$-*c* | pollen transmission | 30 | |
| *sh-c* | by difference | 4 | and 3 |
| *Wx-W* | $F_2$-repulsion | 7 ± 1 | 3.5 |
| | $F_2$-coupling | < 1 | |
| | Eyster (1934) for $w_{11}$ | 1 | |
| $Ga_{8-3}$-*W* | pollen transmission | 2.5 | |

Besides the results reported in this paper some data had also been obtained from the genotype *Bz Ga wx*/*bz ga Wx* backcrossed to *bz Ga wx*/*bz Ga wx*; although the size of the sample was reduced, the distance between *bz* and *Ga* turned out to be about 10, whereas the region *Ga-wx*, from the same data (Schwartz material), was 18.8 c.o. units. Two ears segregating *sh* and *bz* in repulsion with the Bianchi gametophyte factor gave practically the same excess for the two markers (33—34).

Considering all the data so far obtained the position of the *Ga*-factors in respect to the other genetic markers should be tentatively as follows: —

$$C\ 3.5\ sh\ 2\ bz\ 20\ Ga_{8-2 \text{ and } 8-3}\ 0(?)\ Ga_{8-1}\ 2.5\ w_{11(?)}\ 3.5\ wx\ .$$

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* A *w* mutant, ($w_{11}$), at one unit distance from *wx*, was reported by Eyster (1934).

## Zusammenfassung

Innerhalb eines Zeitraumes von 10 Jahren haben wir durch Selbstbestäubung von Pflanzen, die aus normalem Saatgut von geselbsteten *Ga Wx/ga wx*-Genotypen stammten, 54 Kolben mit ca. 25% *wx*-Maiskörnern und 600 Kolben ohne oder nur mit einem sehr kleinen Prozentsatz (ca. 4%) *wx*-Körnern erhalten. Anhand dieser Zahlen kann eine Crossing-over-Rate von ca. 8.7% für die Entfernung zwischen dem *Wx*- und dem durch den älteren Autor dieser Arbeit gefundenen *Ga*-Faktor errechnet werden.

Das gleiche Vorgehen führt für die Repulsionsphase *Ga wx/ga Wx* (die erstmalig und unabhängig von einander von Schwartz und Salamini erhalten wurde) zu einer Schätzung von ca. 13% als c.o.-Entfernung zwischen *Wx* und *ga* (555 Kolben mit einem großen Überschuß an *wx*, 152 mit 25% *wx*-Körnern, 105 Kolben mit keinen und 5 Kolben mit nur wenigen *wx*-Körnern). Der gleiche Faktor war zwischen *Wx* und *Bz* bei ungefähr $^2/_3$ der *Wx-Bz*-Entfernung von *Wx* lokalisiert worden.

Selbstungen von *Ga sh C/ga Sh c*-Pflanzen ergaben einen Überschuß von *sh* (etwa 36.3%) und ein Defizit für *c* (15.3%). Wenn angenommen wird, worauf andere Ergebnisse hindeuten, daß *ga*-Gameten nicht funktionsfähig sind, lassen diese Zahlen für *ga* eine Entfernung von etwa 27 und 31 c.o.-Einheiten von *sh* bzw. *c* vermuten. Eine Entfernung der gleichen Größenordnung von *sh* wurde an Hand von Selbstungen des *Ga Sh/ga sh*-Genotyps festgestellt, die 12% *sh*-Körner ergaben.

Ein zusätzliches Markierungsgen in Chromosom 9, das eng mit *ga* gekoppelt ist, ist ein Albinosämlingsfaktor (*w*). Die vorliegenden Daten zeigen, daß der Albino-Faktor enger bei *Ga* als bei *wx* liegt; sie erlauben auch eine Schätzung des c.o.-Wertes zwischen *wx* und *w* (ca. 2 ± 0.2%).

Falls die *ga*-Gameten funktionsunfähig sind, ergibt sich aus dem verdoppelten Prozentsatz an *w*-Sämlingen (2 × 2% = 4%) ein Schätzwert der Crossing-over-Häufigkeit zwischen *w* und *ga*.

Die Distanz *wx-w* variierte wiederum zwischen 0 und 7 Crossing-over-Einheiten.

Als Koppelungskarte der im kurzen Arm von Chromosom 9 untersuchten Gene ergibt sich auf Grund der vorliegenden Befunde folgende Genfolge:

C 3.5 *sh* 2 *bz* 20 $Ga_{8-2 \text{ und } 8-3}$ 0(?) $Ga_{8-1}$ 2.5 $w_{11\,(?)}$ 3.5 *wx* .

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# Die mutagene Wirkung von Äthylmethansulfonat bei der komplexheterozygoten *Oenothera berteriana*

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### The Mutagenic Effect of Ethylmethane Sulfonate on the Complex Heterozygote *Oenothera berteriana*

**Summary.** Seeds of the complex heterozygote *Oenothera berteriana* were treated in different ways with ethylmethane sulfonate (EMS). Treatment with a 1% solution caused absolute lethality. After the application of a 0,1% solution, however, all surviving plants of the $M_1$-generation were mutants. There is no doubt, that the mutagenic effect of EMS exceeds that of *X*-rays. This is also confirmed by the higher number of viable mutants in the following generation.

While there are mainly translocations among the $X_1$-mutants following *X*-radiation, after EMS-treatment there are no translocations at all. EMS is just causing small, merely visible aberrations. With regard to the kind of the chromosome mutations we can state a clear difference between *X*-radiation and EMS treatment.

In the progeny of $X_1$-mutants caused by *X*-rays there are trisomic plants in a great number, whereas in the progeny of diploid EMS mutants trisomic forms are absolutely absent. So the assumption could be confirmed that translocated chromosomes are responsible for the increasing of non-disjunction, which finally leads to the occurrence of trisomic plants.

## I. Einleitung

Bei unseren mehrjährigen Versuchen zur Induktion außerkaryotischer Mutationen an *Oenothera berteriana* wurde neben vielen anderen chemischen Mutagenen