

5. Relation of multiple chromosome associations at diakinesis to knob number.

Previous studies (S. E. Zvingilas, MGCNL 35 and 36) indicated that the knob and centromere associations of non-homologous chromosomes observed at pachytene persist through metaphase.

Additional investigations were carried on with diakinesis cells of plants which arose from a backcross of the 12 knob heterozygote to the knobless parent. These results support the previous conclusion; there is a positive correlation between the number of chromosomes in multiple association at diakinesis and the number of knobs present (Table 1).

Table 1.

No. of knobs	Ave. no. of assoc. bivalents at diakinesis
1	1.46
2	1.75
3	1.48
4	1.94
5	2.15
6	2.38
7	3.12
8	3.15

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1. Allele studies involving \underline{Cl}_2 , \underline{Cl}_3 and \underline{Cl}_4 .

Dr. Everett in 1949 (Proc. Nat. Acad. Sci. 35: 628-634) described two dominant suppressors of the \underline{cl}_1 locus, \underline{Cl}_2 and \underline{Cl}_3 , which partially or completely suppressed the albino phenotype of this mutant. Since the discovery of the two original suppressors, we have found a third one which has been designated \underline{Cl}_4 . This suppressor was found in a stock in which a gene for albinism was segregating, designated \underline{cl}_p . This gene was found to be allelic to \underline{cl}_1 . The action of the suppressors, \underline{Cl}_2 , \underline{Cl}_3 and \underline{Cl}_4 is summarized in Table I.

Table I. Phenotypes produced by the suppressors \underline{Cl}_2 , \underline{Cl}_3 and \underline{Cl}_4 .

Genotype	Endosperm Phenotype	Seedling and Mature plant phenotype
1. $cl_1 cl_1$	white or pale yellow	albino
2. $cl_1 cl_1 \underline{Cl}_2 cl_2$	white or pale yellow	pale green (pastel) - lethal
3. $cl_1 cl_1 \underline{Cl}_2 \underline{Cl}_2$	white or pale yellow	pale green (pastel), on the average darker green than #2. - lethal
4. $cl_1 cl_1 \underline{Cl}_3 cl_3$	white or pale yellow	green as seedling, mature plant pale green, often zebra
5. $cl_1 cl_1 \underline{Cl}_3 \underline{Cl}_3$	white or pale yellow	green as seedling, green as mature plant apparently as vigorous as normal siblings.
6. $cl_1 cl_1 \underline{Cl}_4 cl_4$	white or pale yellow	green as seedlings - lethal
7. $cl_1 cl_1 \underline{Cl}_4 \underline{Cl}_4$	white or pale yellow	green as seedlings, green as mature plant but not as vigorous as normal siblings

While it was known that the suppressors were inherited independently of the \underline{cl}_1 locus, it was not known whether they occupied the same or independent loci. The following crosses were made to determine this.

1. $\underline{cl}_1 \underline{cl}_1 \underline{Cl}_3 \underline{Cl}_3$ x $\underline{cl}_1 \underline{Cl}_1 \underline{Cl}_2 \underline{Cl}_2$
2. $\underline{cl}_1 \underline{Cl}_1 \underline{Cl}_2 \underline{cl}_2$ x $\underline{cl}_p \underline{cl}_p \underline{Cl}_4 \underline{Cl}_4$
3. $\underline{cl}_1 \underline{Cl}_1 \underline{Cl}_2 \underline{Cl}_2$ x $\underline{cl}_p \underline{cl}_p \underline{Cl}_4 \underline{Cl}_4$
4. $\underline{cl}_p \underline{cl}_p \underline{Cl}_4 \underline{Cl}_4$ x $\underline{cl}_1 \underline{cl}_1 \underline{Cl}_3 \underline{Cl}_3$

Yellow and white or pale yellow seeds from the F_1 ears were planted in the field. The plants from the white or pale yellow seeds of crosses #1 and #4 produced plants that survived to maturity and produced ears. Most of the plants from the white or pale yellow seeds of crosses #2 and #3 died at an early age and the few that did survive were pale green runts. All surviving plants were self pollinated and where possible, a sample of 50 white or pale yellow seeds was planted from each ear. If the suppressors are alleles, no albino seedlings should be observed in the F_2 's of crosses #1, #3 and #4. If the suppressors occupy independent loci, then a ratio of 15 non-albino : 1 albino seedling should be observed among the plants produced by the white or pale yellow seeds from such F_2 ears. A lower frequency of albino seedlings would indicate that the

suppressors are non-allelic but linked. Half of the F₁ plants from cross #2 will have cl₂ and the white seeds produced when these plants are self pollinated, therefore, will be expected to segregate albino seedlings in a 3 green to 1 albino ratio but no pastel seedlings. This was observed to be the case. The albino segregating ears were not included in the totals given in Table II. The results of these tests which are summarized in Table II indicate that these three suppressor genes are allelic.

Table II. Summary of Data from Allele Tests Involving Cl₂, Cl₃ and Cl₄.

Segregating Alleles	Number of Seedlings Tested	Number of Albino Seedlings	Conclusions
<u>Cl</u> ₂ and <u>Cl</u> ₃	4277	0	Allelic
<u>Cl</u> ₂ and <u>Cl</u> ₄	3495	0	Allelic
<u>Cl</u> ₃ and <u>Cl</u> ₄	3604	0	Allelic

The absence of albino seedlings in these F₂ populations also indicates that the cl_p gene for albinism (present in the original Cl₄ stock) can also be suppressed by Cl₂ and Cl₃.

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2. Chromosomal segregation in hyperploid TB-9b plants used as females.

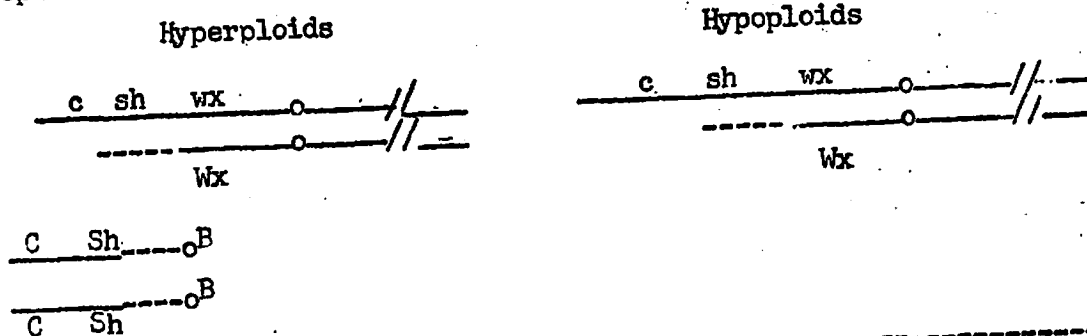
As a regular practice, we have been perpetuating several of our B translocation stocks by using hyperploid plants (e.g., 9 9^BB^B) as males in outcrosses to inbreds. Pollen examination is used to select hyperploid plants for outcrossing but since sterility is low (approximately 15% - 20%), it is not always possible to select these plants by this method. Therefore, each suspected hyperploid is also crossed to appropriate tester stocks carrying recessive genes that are found in the region of the chromosome translocated to the B centromere.

For TB-9b we have been using a c sh₁ wx line as the tester stock. The break point in this translocation is about 40% of the distance out on the short arm of nine and the translocated piece includes the loci of c and sh₁. The wx locus is on the untranslocated portion of nine. When hyperploid plants are crossed as males to this tester stock, two classes of seeds are found on the ears, purple, starchy and colorless, shrunken. The latter class results when a deficient sperm (9^B) unites with the polar fusion nucleus producing endosperm cells with the chromosomal constitution of 9 9 9^B. The egg nucleus of such seeds will have been fertilized by the hyperploid sperm (9^B9^B9^B) resulting in embryos with the chromosomal constitution of 9 9^B9^B9^B. Some of the

purple, starchy seeds will be produced by the reciprocal fertilization and thus will have hypoploid embryos ($9\ 9^B$) and hyperploid endosperms ($9\ 9\ 9^B\ 9^B$). Plants from this latter class of seeds are semisterile. Figure one diagrams the genetic and cytological constitution of the embryos from both of these classes of seeds.

Figure 1

Genetic and cytological constitution of embryos hyperploid and hypoploid for TB-9b.



Plants from both of these classes of seeds have been testcrossed with a $c\ sh_1\ wx$ stock. In the case of the seeds with hyperploid embryos, the crosses were made using the hyperploid plants as females while the hypoploid plants were crossed as both males and females.

Table 1 gives the results for the testcrosses of hyperploid plants. Classes $C\ Sh_1\ Wx$ and $C\ Sh_1\ wx$ are the most frequent and occur in equal frequency. These results would be expected if most of the time the normal chromosome nine paired with 9^B and the two B^9 chromosomes paired and if these two pairs then assorted independently of each other at meiosis I. The next most frequent class is $c\ sh_1\ wx$. This class would be expected if a gamete received only a normal chromosome 9 and could be the result of non-disjunction of the B^9 centromere either in meiosis or during development of the female gametophyte. The latter possibility is not very likely since non-disjunction would have to occur in the two cell lineages giving rise to the two polar nuclei.

In order to get the $c\ Sh_1\ Wx$ class, a B^9 chromosome must have paired with a normal nine followed by crossing over between the c and sh_1 loci which would produce a crossover chromatid of the B^9 chromosome with the genotype $c\ Sh_1$. In order to account for the Wx allele, the 9^B chromosome (carrying wx) must have ended up in the same cell as the B^9 crossover chromatid. It will be noted that none of the reciprocal crossover classes occurred ($C\ sh_1\ wx$) and that with the exception of one seed for which the shrunken classification was

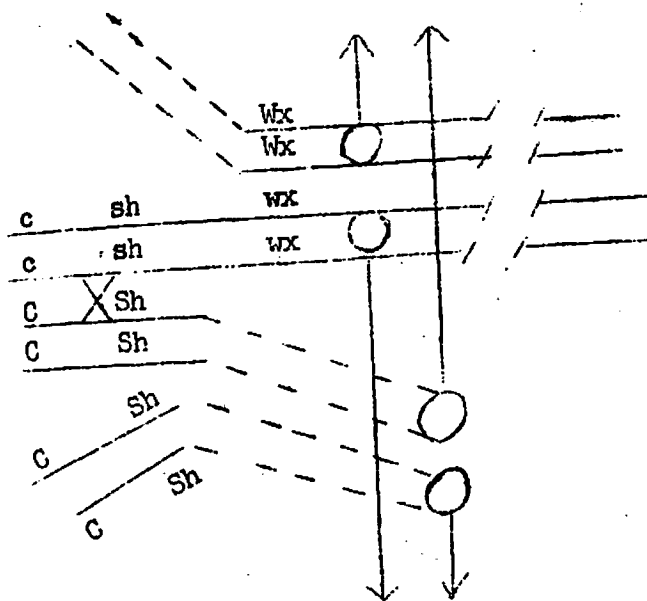
doubtful (a classification which was made and recorded prior to any attempt at interpreting the results), no seeds of the $c \underline{Sh}_1 \underline{wx}$ class were found. This class is theoretically possible if after the above crossover event, the B^9 chromosome and the normal 9 chromosome went to the same pole and if after the second meiotic division, a microspore received the crossover B^9 chromatid ($c \underline{Sh}_1$) and at the same time, the non-crossover 9 chromatid ($c \underline{sh}_1 \underline{wx}$)*. Since neither the $C \underline{sh}_1 \underline{wx}$ nor $c \underline{Sh}_1 \underline{wx}$ are found, the events which are responsible for them as outlined above must be extremely rare and some type of directed segregation must take place whenever a crossover takes place in the $c - \underline{sh}_1$ region. Figure 2 diagrams the pairing relationships and segregations necessary to explain the observed results. The results indicated that a crossover in the $c - \underline{sh}_1$ region determines that the normal chromosome 9 and the B^9 chromosome involved in the crossover will go to opposite poles. This segregation then determines the poles to which the other centromeres will move with homologous centromeres going to opposite poles. This observation is in agreement with that of Burnham (Genetics 35: 446-481, 1950) where he found that chromosomes involved in a crossover in the interstitial region of a heterozygous translocation passed to opposite poles.

Table 1. Testcross data of plants hyperplod for TB-9b ($c \underline{sh}_1 \underline{wx}/C \underline{Sh}_1 -/C \underline{Sh}_1 -/--\underline{wx}$).

	CShwx	cshwx	Cshwx	cShwx	CShwx	cshwx	Cshwx	cShwx
<u>5561-4</u> 4558-4	112	5	-	-	137	3	-	-
<u>5561-5</u> 4558-4	221	15	-	1	237	3	-	-
<u>5561-6</u> 4558-4	160	8	-	-	152	1	-	-
<u>5561-16</u> 4558-4	243	16	-	1	242	-	-	-
<u>4564-2</u> 4558-3	284	31	-	1	271	-	-	-
<u>4564-16</u> 4558-3	290	27	-	1	276	-	-	(1)
Totals	1310	102	-	4	1315	7	-	(1)
%	47.8%	3.7%	-	0.1%	48.0%	0.26%	-	(0.04%)

*Theoretically, the $c \underline{Sh}_1 \underline{wx}$ class also could be produced by a double crossover between the normal 9 chromosome and the B^9 chromosome which would move \underline{Sh}_1 into a normal 9 chromatid. If the crossover were then followed by non-disjunction of the B^9 chromosomes, some gametes of the $c \underline{Sh}_1 \underline{wx}$ would be expected. Since this would involve the simultaneous occurrence of two rare events (double crossover and non-disjunction), it is not likely that this genotype would be produced in this manner.

Figure 2. Probable pairing relationships and segregation responsible for the c Sh₁ Wx class.



Three events could give rise to the c sh₁ Wx class. 1) A crossover could take place between the wx locus and the translocation point putting wx on the 9 chromosome along with c sh₁ and this event accompanied by non-disjunction of the B centromeres, 2) A crossover could have taken place between the sh₁ locus and the translocation point putting c and sh₁ on the B⁹ chromosome, which as the data from the c Sh₁ Wx class indicate would then segregate from chromosome 9, ending up in a cell with the 9B chromosome carrying wx or 3) Non-disjunction of the 9 centromeres and B centromeres, in which the normal 9 and 9B chromosomes go to one pole and the two B⁹ chromosomes to the other.

Table 2 gives the results of testcrosses of deficient plants. From these data, the amount of crossing over between the waxy locus and the translocation break point has been determined to be .48%. This information permits the elimination of one of the three explanations for the c sh₁ Wx class given above. If explanation 1 is responsible for this class, the frequency with which these seeds can be expected can be predicted. This predicted value should be equal to the probability of non-disjunction (=2 x .037, the frequency of the c sh₁ wx non-disjunctional class) times the probability of a crossover between the wx locus and the translocation break point (.0048). Since there are two ways the non-disjunctional chromosomes will segregate

with respect to the crossover products and only one will allow for the expression of the $c\ sh_1\ Wx$ genotype, this product will have to be divided by 2. Further, if the desired non-disjunction takes place in the first division of meiosis, only three of the meiotic products will be functional and of these, only one will carry $c\ sh_1\ Wx$. This requires that the above product be divided by an additional factor of 3. However, if the non-disjunction takes place in the second division, 1/2 of the products following non-disjunction will be $c\ sh_1\ Wx$ and, therefore, instead of dividing by 3, the second division should be by 2. To summarize, the formula will be:

$$\frac{(\text{Probability of C.O.}) \times 2 \times (\text{probability of } c\ sh_1\ wx)}{2 \times (3 \text{ or } 2) \text{ depending upon when non-disjunction occurs}} =$$

$$\frac{.0048 \times .037}{3(\text{if first division non-disjunction})} = .00006 \text{ or } \frac{.0048 \times .037}{2(\text{if 2nd division non-disjunction})} = .00009$$

Since both of these predicted values are considerably less than the observed frequency of .0026 for the $c\ sh_1\ Wx$ class, it seems likely that explanation 2 or 3 is responsible for most of the seeds of this phenotype. Explanation 2 is most probable since explanation 3 requires the occurrence of two rare non-disjunctions, one of which has not been demonstrated to take place (i.e., the 9 centromeres).

The results of these studies should be taken into consideration in using B translocations, particularly if one is attempting to incorporate genes into the translocated piece. The likelihood of succeeding will be extremely low if this is attempted in a hyperploid. However, if a hyperploid is outcrossed as a female, approximately half of the offspring will then be heterozygous for the translocation (9 9BB9), a condition which would be more conducive to picking up the desired crossovers.

Table 2. Testcross data of plants hypoploid for TB-9b ($c\ sh_1\ wx/--wx$).

	cshwx	cshwx		cshwx	cshwx
$\frac{4558-8}{4893-9}$	1	174	$\frac{4894-2}{4893-3}$	0	207
$\frac{4558-13}{4893-9}$	0	97	$\frac{4894-5}{4893-3}$	0	217
$\frac{4893-2}{4894-3}$	0	130	$\frac{4894-8}{4893-9}$	1	216
$\frac{4893-2T}{4894-3}$	1	160	$\frac{4895-1}{4893-9}$	4	160
$\frac{4893-3}{4894-4}$	0	189	$\frac{4895-3}{4893-3}$	3	326
$\frac{4893-9}{4558-13}$	0	183	Total	10	2059
			% C. O.	.48%	

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