2. <u>Studies with overlapping inversions</u>.

Cytological and genetical studies with In 3a have been presented in a paper published in the Amer. J. Botany (1953). This is a paracentric inversion with both breaks in 3L; the proximal break is at .4 and the distal break at .9. If the long arm is divided into 11 equal segments with segment 1 nearest the centromere and segment 11 representing the distal segment, the proximal break in In 3a lies between segments 4 and 5 and the distal break just to the right of segment 10. Evidence was given that the Lg_2, A_1, and Et loci are included in the inverted segment while the Rg locus was in the proximal uninverted region. That both Rg and Gl_6 lie in the proximal uninverted segment is clear from the following B. C. data from plants homozygous for In 3a.

In gl A Lg/ In Gl a lg   x gl lg a

(0) gl A Lg	275	(2) gl A lg	174
(0) Gl A lg	254	(2) Gl a Lg	158
(1) gl a lg	71	(1-2) gl a Lg	27
(1) Gl A Lg	64	(1-2) Gl A lg	40

gl-A = 19.0% recombination
A-Lg = 37.5% "
gl-Lg = 43.9% "
The order is gl A lg

In rg a lg/ In Rg A Lg   x rg lg a

(0) rg a lg	88	(2) rg a Lg	58
(0) Rg A Lg	76	(2) Rg A lg	71
(1) rg A Lg	31	(1-2) rg A lg	24
(1) Rg A lg	25	(1-2) Rg a Lg	24

Rg-A = 26.2% recombination
A-Lg = 44.6% "
Rg-Lg = 46.6% "
The order is Rg A lg

Since Rg-Gl_6 shows approximately 5% recombination, it would appear that the normal order is Rg-Gl_6-lg-A-et but the vagaries of recombination values are such that this order is highly uncertain and may be gl-Rg-lg-A-et. That this latter order may be correct is suggested by the following data obtained from sib plants of the family having 26.2% recombination between Rg and A.

In Gl a lg/ In gl A Lg   x gl lg a

(0) Gl a lg	43	(2) Gl a Lg	33
(0) gl A Lg	48	(2) gl A lg	55
(1) Gl A Lg	22	(1-2) Gl A lg	23
(1) gl a lg	19	(1-2) gl a Lg	13

Gl-A = 30.1% recombination
A-Lg = 48.4% "
Gl-Lg = 50.4% "

This is a small population but the recombination value for Gl-A is greater than that for Rg-A. Three point tests are underway to determine the correct order and they should be completed by this summer.

Another paracentric inversion in 3L is the one found by Longley and designated as In 3b. The proximal break is between segments 2 and 3 and the distal break between segments 8 and 9. In 3a is 1 2 3 4 10 9 8 7 6 5 11 while In 3b is 1 2 8 7 6 5 4 3 9 10 11 so the two have a common inverted segment 8 7 6 5 -- i.e., they are overlapping inversions. Backcross data for In 3b heterozygotes are presented below from the cross of:

In Gl Lg A Et/ N gl lg a et   x N gl lg a et

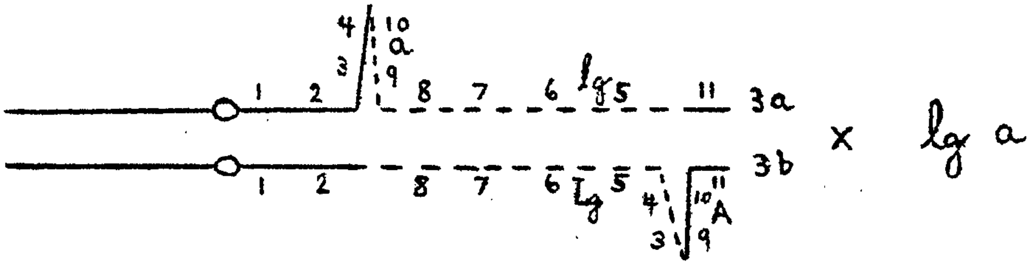
Gl Lg A Et	1527	Gl Lg a Et	6
gl lg a et	777	gl lg A et	5
Gl Lg A et	184	Gl Lg a et	90
gl lg a Et	358	gl lg A Et	194
gl Lg A Et	3	Gl lg A et	2
Gl lg a et	5	gl Lg a Et	0
Gl lg A Et	3	Gl lg a Et	3
gl Lg a et	2	gl Lg A et	0

(The marked discrepancy in numbers between the complementary classes is due to the semi-lethal action of the et allele.)

	In/N	Control
Gl-lg	0.57%	29%
Gl-A	9.7%	49%
Lg-A	9.6%	36%
A-Et	17.7%	12%

The A locus is not included in In 3b although certainly Lg_2 and probably Gl_6 are. If Gl_6 is not included, it must lie very close to the proximal break point in terms of recombination units. The increased recombination value for the distal A-Et region in In 3b heterozygotes is in agreement with studies on other inversions which have shown higher than normal crossover values for both proximal and distal uninverted regions in inversion heterozygotes. This finding is contrary to the data obtained from Drosophila inversions.

Inasmuch as In 3a and In 3b are overlapping inversions, crosses were made to obtain plants carrying both inversions. Crossing over in the common inverted segment should lead to a chromosome with a known duplication and to one deficient strand. The In 3a chromosome had the recessive a_1 and lg loci, both of which lie in the inverted region, while the In 3b chromosome carried the dominant alleles but only the Lg locus is in the inversion since A_1 lies distally.



The common inverted segment is 8 7 6 5 with the lg locus lying between 6 and 5. Single crossovers in this segment, to the left of lg give a Lg A chromosomes and a complementary lethal combination. These a Lg A crossovers cannot be distinguished without breeding tests from noncrossover Lg A chromosomes but would lead to an excess of A over a gametes. Single crossovers in this common segment to the right of lg produce a lg A chromosomes. These are 1 2 3 4 10 9 8 7 6 5 4 3 9 10 11 in constitution. The complementary crossover class is not recovered. Thus all single exchanges within the common inverted segment lead to viable A-bearing duplication chromosomes; the complementary crossover class is lethal. The excess of A over a kernels is a measure, although obviously not the most precise one, of the frequency of single exchanges in the 8 7 6 5 region. A total of 8529 A: 7466 a kernels were obtained. The difference of 1063 is due to single exchanges producing a chromosome 3 with both the a and A alleles. The total number of crossover chromosomes is 2 x 1063 or 2126 which gives a frequency of single exchanges in the 8 7 6 5 region of 12.5 percent.

Double crossovers with one exchange to the left of lg and one to the right yield In 3b chromosomes with the lg and A alleles and the complementary crossover class which is In 3a and carries the a and Lg alleles. Both are viable since neither has a deficiency or a duplication.

A portion of the ears included in the above total were planted and gave the following data:

A Lg	A lg	a Lg	a lg
3560	92	10	3092

$\sum = 6754$

By taking into consideration the inviable gametes, these data may be converted as follow:

(0)	Lg A	In 3b	3092	viable
(0)	a lg	In 3a	3092	viable
(1)	a Lg A	In Dp	468	viable
(1)	lg	In Df	468	inviable
(2)	a lg A	In Dp	82	viable
(2)	Lg	In Df	82	inviable
(1-2)	a Lg	In 3a	10	viable
(1-2)	lg A	In 3b	10	viable

The amount of recombination in the 8 7 6 5 segment may be broken down as follows:

	singles		doubles		recombination value
(1)	12.8%	+	.3%	=	13.1%
(2)	2.2%	+	.3%	=	2.5%

The total frequency of single crossovers in both regions (15.0%) agrees fairly well with the value of 12.5% obtained from the more extensive kernel data. In structurally normal plants this segment would have a map distance of approximately 40 units. It is clear, therefore that the structural dissimilarity to the left and to the right of the 8 7 6 5 segment in In 3a/In 3b heterozygotes markedly reduces crossover values for this segment.

Even though a reduction in crossing over occurs in the 8 7 6 5 segment there is some evidence that the frequency of double crossovers is high when pairing does occur for this region. The expected number of double crossovers with no interference is (.131) (.025) (7304) = 24 individuals and the observed number of doubles is 26, giving a coincidence value of 1.1.

The great majority of A lg plants derived from In 3a/In 3b heterozygotes of the constitution diagrammed above should possess the 1 2 3 4 10 9 8 7 6 5 4 3 9 10 11 segments in this order. Nine A lg plants were examined cytologically and eight had the inverted order of In 3a with a duplication of the 4 3 9 10 segments. These came from single crossovers to the right of lg. The remaining A lg plant had the In 3b chromosome and came from a double exchange to the left and right of lg. Four a Lg plants were studied cytologically and all had an In 3a chromosome as expected from a double crossover origin.

The A lg plants coming from single crossovers should carry the recessive a allele in the proximal 10 9 segment, the recessive lg allele in the 6 5 segment and the dominant A allele in the 9 10 segment of the duplication. Chromosomes of this constitution will be referred to as In Dp a lg A. That the recessive a allele is cryptically carried by the a lg A chromosome is evident from the following tests.

Plants homozygous for the In Dp a lg A chromosome have bred true for aleurone color although only limited tests have as yet been made. Crosses were made to obtain heterozygotes for the In Dp a lg A and N Lg A chromosomes. When these heterozygotes were test-crossed by a plants, .2% of the resulting kernels were colorless while 99.8% were colored. These infrequent colorless kernels arise from 2 and 3 strand double exchanges within the inversion loop producing a chromosome 3 with the a allele. Their rare occurrence would be attributed to a mutational phenomenon of some sort if the structural composition of the chromosomes were not known.

When several plants of In Dp a lg A/ N Lg a-x_1 constitution were self-pollinated there were 21 colorless kernels in a population of 493. Homozygous a-x_1 zygotes are lethal so the appearance of a viable a class is due to crossing over following pairing of the uninverted distal 9 10 11 segments of the two homologues thus transferring a-x_1 to the In Dp chromosome and producing a viable *a lg a-x*$_1$ chromosome. The colorless kernels should be In Dp *a lg a-x*$_1$/ N *Lg a-x*$_1$. Plants of In Dp *a lg A*/ In Dp *a lg a-x*$_1$ constitution gave a ratio of 1A:1a when used as the pollen parents in test-crosses. The deleterious effect of *a-x*$_1$ is thus covered by the presence of the *a* allele lying in the same chromosome.

In Dp chromosomes are transmitted normally through the megaspores but In Dp pollen is at a disadvantage in competition with N pollen. This is evident in the cross of *lg a* x In Dp *a lg A*/ N *Lg A* which produced 2354 colored and 5 colorless kernels. The 2354 colored kernels yielded 1818 *Lg* and 429 *lg* (19.1%) seedlings. Most of the *lg* class came from the functioning of In Dp pollen. Crosses of *lg a* x In Dp *a lg a*/ N *Lg A* gave the following phenotypic classes:

A Lg	A lg	a Lg	a lg	
840	58	186	279	∑ = 1363
(61.6%)	(4.3%)	(13.6%)	(20.5%)	

Save for very infrequent double exchanges within the inversion loop both the *A lg* and *a lg* classes come from In Dp pollen. The *A lg* class has an *a lg A* chromosome derived from crossing over in the distal 9 10 11 segments.

A somewhat similar cross of *lg a* x In Dp *a lg A*/ N *Lg a* gave the following phenotypic classes:

A Lg	A lg	a Lg	a lg	
316	525	1425	95	∑ = 2361
(13.4%)	(22.2%)	(60.4%)	(4.0%)	

As in the above cross the *A lg* and *a lg* classes are derived almost exclusively from the functioning of In Dp pollen. The *a lg* class has an In Dp *a lg a* chromosome derived from crossing over in the distal 9 10 11 segment. From the data of the above two crosses the frequency of crossing over between the break point of In 3b and the *A* locus is 17.7%. This is a significantly higher value than the 9.6% found for the same region in In 3b/N heterozygotes. The greater structural dissimilarity in In Dp/N compounds as compared to In 3b/N leads to more frequent pairing of the uninverted distal 9 10 11 segment and hence to higher recombination values.