On the Cross-over and Interference in the Japanese Morning Glory.

By

Tokio Hagiwara.

Outside of *Drosophila*, the only known case of the interference of cross-over is that of *primula sinensis* reported by ALTENBURG.[1] In the course of my genetic studies on the Japanese Morning Glory (*Pharbitis Nil*), I have observed the phenomena of the double crossing-over and interference, and the results of my experiments will be given in the following lines.

Linkage Group

I have reported in the "Journal of the Scientific Agricultural Society No. 273, 1921" that three factors of the leaf, variegated (v), rolled (d) and heart-shaped (h) make a linkage group. The variegated (v) is a simple Mendelian recessive to the normal. The rolled (d) is a simple Mendelian recessive to the flat. I have reported the relation between v and d in the "Botanical Magazine No. 399, vol. XXXIV 1920." I obtained the F_1 plants having the genotype VvDd from the cross VVDD × vvdd. The total sum of the separation numbers of F_2 and F_3 is as follows:

	VD	Vd	vD	vd	Total
F_2 experimental numbers	498	51	48	135	733
F_3 experimental numbers	255	27	28	74	384
Total experim. numbers	753	78	77	209	1117
Theoretical numbers	767.325	70.395	70.395	208.885	1117

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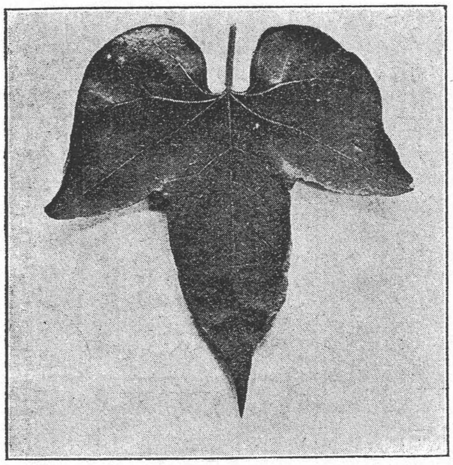
1) American Naturalist, Vol. LV, No. 636, 1921.



Rolled and normal leaf.

The actual numbers nearly coincide with the theoretical numbers, when the F_1 plants' gametic ratio is calculated as 6.4 : 1 : 1 : 6.4. Thus, the cross-over percentage between v and d is 13.51 %. The heart-shaped leaf (h) is a simple Mendelian recessive to the normal (three lobed leaf). I obtained the F_1 plants having genotype VvHh from the cross VVHH × vvhh. From these F_1 plants were produced the offspring shown in the following table.

	HV	Hv	hV	hv	Total
F_2 experim. numbers	736	214	221	112	1283
Theoretical numbers	750.638	211.607	211.607	109.148	1283

When the gametic ratio of the F_1 plants is calculated as 1.4:1:1:1.4 the theoretical number almost agree with the experimental numbers. Thus I would recognize that this ie also a case of coupling between the factors h and v, although its intensity is low. Hence, the cross-over is 41.67%. Recognizing the linkage between v and d, and between h and v, respectively, a linkage should be found between h and d. I have also proved that there is a linkage between the factors h and d by the experimental crosses DDHH × ddhh, the results of of the segregation being as in the following table:

	DH	Dh	dH	dh	Total
F_2 experim. numbers	537	182	177	82	1028
Theoretical numbers	596.303	175.096	175.096	82.505	1028

When the gametic ratio of F_1 plants is calculated as 1.3:1:1:1.3 the theoretical numbers almost agree with the experimental numbers. Thus, the cross-over percentage between h and d is 43.48%.

The three factors d,h, and v constituting a linkage group, may be considered to be arranged on the same chromosome. The distance between v locus and d locus is 13.51, that between v and h 41.69, and between d and h 43.48. If the order of loci on the chromosome is as h, v, d, or h, d, v, the distance between h and d should be

55.29. (i. e., 13.51+41.69) or 28.18 (i.e., 41.69–14.51), respectively. As the distance between h and d obtained by my experiments is 43.48, the order of these factors should be d, v, h.

Double Crossing-over and Interference.

As stated above the loci of these factors are arranged on the chromosome in the order h, v, d. The calculated distance between h and d is 55.20 while the experimental distance is only 42.19. The difference is 13.01. What is the cause of such difference? By a three point experiment, MORGAN[1] and others recognized that the calculated distance is longer than the experimental distance, especially in the case of distance being longer and that such difference is due to the double crossing-over.

In the germ cell of the F_1 plants produced by the conjugation of two different germ cells which have the factors D, V, H and d, v, h respectively, two kinds of chromosomes should be found as illustrated in Fig. I a, b. In the germ cell of F_1 plants, the cross-over take place between the homologous chromosomes.

Considering the arrangement of these three factors v, d, and h, all kinds of cross-overs are illustrated in Figures II,IV and VI. Fig. II is the cross-over between V and D, and Fig. IV between V and H. These cross-overs are called the single cross-overs. From the former results two chromosomes as illustrated in Fig. III, the one having the three factors, not rolled, variegated and heart-shaped and the other one rolled, not variegated and not heart-shaped. From the latter results two chromosomes as illustrated in Fig. V, the one having respectively three factors not rolled, not variegated and heart-shaped and the other one rolled, not variegated and heart-shaped on the one hand, and rolled, variegated and not heart-shaped on the other. As illustrated in Fig. VI, there is still a case in which the cross-over occurs at two points, i. e., between D and V, and V and H respectively. This cross-over is called the double crossing over and there is formed two chromosomes one of which has three factors, not rolled, variegated and not heart-shaped, and the other not variegated, rolled and heart-shaped as illustrated in Fig. VII. There is, of course, another case in which no cross-over occurs as illustrated in Fig. I a, b. In short, F_1 plants must give eight kinds of individuals having the three characters different from each other.

1) The Mechanism of Mendelian Heredity. 1915.

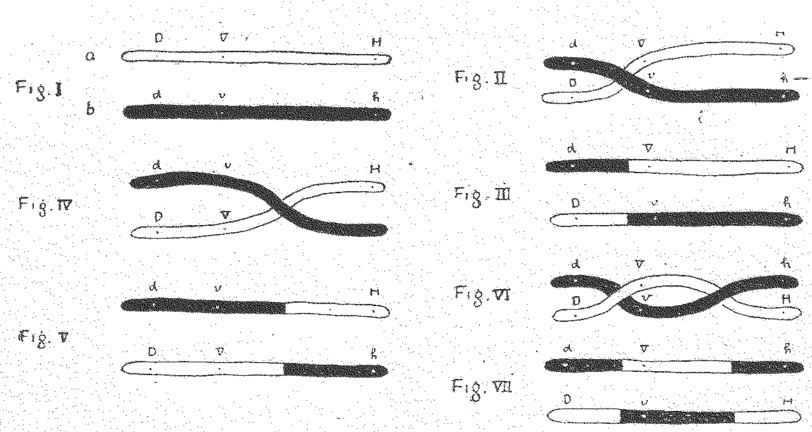


Fig. I a. The diagram of a chromosome having the factors V,D,H.
Fig. I b. The diagram of the homologous chromosome having the factors v. d, h.
Fig. II. Single cross-over occurring between D,V.
Fig. III. One couple of chromosomes after the breaking took place in Fig. II.
Fig. IV. Single cross-over occurring between V and H.
Fig. V. One couple of chromosome after the breaking took place in Fig. IV.
Fig. VI. Double crossing-over.
Fig. VII. One couple of chromosomes after the double crossing-over.

This statement is confirmed by the following experiments. After the maturation in the germ cells of the F_1 plants, we can verify the presence of the eight kinds of chromosomes by back crosses (See TABLE I). Although my experimental numbers were small compared with others, I can see clearly the eight kinds of individuals each having any one of the above mentioned eight kinds of chromosomes through the back cross experiments that the homogeneous races designate as the 5 having the factors v, d, h cross with the F_1 plants which were obtained from the cross between the 5 and the homogeneous races designate as the 9 A having the alleromorphic factors V, D, H, and also having the reciprocal of the above crosses.

TABLE I

	non-crossovers		crossovers						
			single				double		
	DVH	dvh	Dvh	dVH	DVh	dvH	DvH	dVh	total
B.C. (5×9A)×5	42	36	4	2	24	33	6	5	152
B.C. 5×(5×9A)	4	4	1	1	0	3	1	3	17
Total	46	40	5	3	24	36	7	8	169
			8		60		15		
			4.8 %		35.5 %		8.9 %		

The total amount of the cross-over between V and D is the sum of the single (4.8) and of the double (8.9) cross-over, of which the value is 13.7. Likewise the cross-over value for V, H, is $35.5+8.9=44.4$.

If the cross-overs occurred independently of each other, the double crossing-over involving both regions D-V, V-H, should take place in 6.08 percent of the cases. In other words, the expected double crossing-over without the occurring of interference should be $13.7\times44.4/100=6.08\%$. Yet the experimental cross-over is 8.9 %. Therefore, the difference between the expected double crossing-over and the experimental must be due to the interference of the cross-overs.

The phenomenon of interference was originally deduced by STURTEVANT[1] from his experiment with *Drosophila melanogaster*. It consists of the fact that the occurrence of a cross-over in one region of a chromosome reduces the chance for the occurrence of another cross-over in a different region of the same chromosome. Studying mathematically this phenomenon MULLER[2] said that the amount of interference is expressed by an index called a "coincidence," which is the ratio of the double crossing-overs actually observed in the experiment as compared with that expected.

It has been recognized that increasing the distance between the two regions in whch the double crossing-over occurred, the coincidence rises gradually to the value of 1, and that when the distance becomes so great that the coincidence reach the value of 1 the interference has entirely disappeared, or the expected double crossing-over have become equal to the experimental. According to the further studies of the phenomenon by MULLER[3], BRIDGES[4], and WEINSTEIN[5], when the two regions are widely separated the intereference reappears. In this case there is actually found a greater number of double crossing-overs than the expected.

In his experiment concerning three factors, black, purple and curved, locating on the second chromosome of *Drosophila melanogaster*, BRIDGES reported that the experimental double crossing-over is larger than the expected. BRIDGES said that in his case although the difference between the experimental and the expected is small, it

1) Journal of Experimental Zoology, Vol. XIV, 1913.
2) American Naturalist, Vol. L, No. 593, 1916.
3) American Naturalist, Vol. L, No. 593, 1916.
4) Journal of Experimental Zoology, Vol. XIX, 1915.
5) Genetics, Vol. III, No. 2, 1918.

is not due to a chance fluctuation, but to the interference to which he gives especially the name of "Negative interference."

I consider that the interference which occurred in my experiment is to be classified as "Negative interference." I have found that in my case the coincidence is 1.46.

Comparing the few examples of the coincidence which were obtained by other investigators with mine, it will be tabulated as follows.

Materials	Characters	Double crossing-overs		Sum of the distance of two regions	Coincidence	Authors
		Observed	Expected			
Drosophila melanogaster	vermilion sable bar	0.28	1.28	23.58	0.21	Morgan[1]
Drosophila melanogaster	purple black curved	1.07	0.97	23.46	1.11	Bridges
Drosophila simulans	yellow rubyoid tiny-bristle	5.08	6.27	57.5	0.81	Sturtevant[2]
Drosophila simulans	rubyoid forked tiny-bristle	0.31	0.24	41.2	1.27	"
Primula sinensis	red stigma long style red flower	2.9	4.1	47.8	0.71	Altenburg
Pharbitis Nil	variegated rolled heart-shaped	8.9	6.08	58.1	1.46	Hagiwara

In concluding, I must express my hearty thanks to Prof. TAKASHI SASAKI who has given me valuable helps during my experiments, and also to Dr. KIICHI MIYAKE and Mr. YOSHITAKA IMAI who were very helpful in writing this paper.

AGRICULTURAL COLLEGE,
IMPERIAL UNIVERSITY OF TOKYO.

1) Sex-linked inheritance in *Drosophila*. Carnegie Pub. No. 737, 1916

2) Genetics, Vol. VI. 1921.