Fixation of Genes and Breakup of Linkage Blocks in Selfed Generations

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Occurrence of meiotic crossover and transmission of recombined chromosomes in selfed generations after a cross of two pure lines were simulated by applying the Monte Carlo method and the changes in the magnitude of heterozygosity and in the length of parental linkage blocks with the generations were examined. The number of generations required for the fixation of all the genes on a chromosome was proportional to the logarithm of the number of crossovers per chromosome (NCR). Increase in NCR from 1 to 10 resulted in the delay of fixation generation from 4.25 to 7.32. A cumulative distribution curve with $NCR=z$ for the frequency of chromosomes homozygous for the whole length was roughly equivalent to the curve for independent factors with the number of factors equal to z multiplied by 3.3 to 4.0. The number of generations needed for the fixation of the whole genome depended upon the number of pairs of homologous chromosomes involved in the genome as well as NCR. When $NCR=2$, for instance, the generation when 95% or more of the individuals come to complete fixation is F_9 for a single pair of chromosomes, while F_{12} and F_{13} for a set of 7 and 12 pairs, respectively. Delay of fixation generation with increase of NCR was similar irrespective of the number of chromosomes. The average number of breakups of the linkage blocks increased from 0.95 to 7.31 in proportion to the increase in NCR from 1 to 10 and the maximum number ranged from 5 to 17. For a particular value of NCR, there was a significant positive correlation between the number of generations needed for fixation and the number of breakups. When 1% of the individuals with the highest number of breakups was selected, the average number of breakups was 2 ($NCR=1$) to 5 ($NCR=10$) times that of the original, while the number of generations needed for fixation changed into F_9 or so irrespective of NCR. Increase in NCR only in a single generation was effective in raising the number of breakups when the increase occurred in early generations. It was found moreover by simulation that the fixation of the whole genome in as early as F_2 by applying the technique of anther culture and doubling of chromosome number resulted in a narrower distribution of the number of breakups and highly recombined genotypes could not be obtained.

KEY WORDS : Selfing, linkage, crossover, fixation, simulation, anther culture.

Introduction

To improve self-pollinated crops by hybridization, recombination of parental genes to provide materials for selection of desired genotypes and fixation of the recombined genome after selection are of vital importance and theoretical evaluations of the problem have so far been repeatedly performed.

In most of the studies, however, the problem has been solved in relation to a small number of loci and not to the whole genome or a large number of loci. The rate of progress toward complete homozygosity and the frequency of recombined genotypes in selfed generations is, if the genes are unlinked, quite easily obtained by applying the formula of JONES (1918). Since any of the genes in the genome is more or less linked to others located on the same chromosome, JONES' formula assuming complete independence of the loci can not be applied to the cases where many loci are involved. The frequency of different genotypes at linked loci in selfed generations can be calculated using a

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transition matrix (NELDER 1952, KEMPTHORNE 1957, BULMER 1980) but the matrix is not a simple form unless the number of loci is as small as two or three. Thus, theoretical study of fixation and recombination of a large number of mutually linked loci requires another approach.

HANSON (1959 a, b, c, d) showed that the recombination of the genes on a chromosome can be examined by treating the lineage of the genes in the parents as a block and by mathematically determining how the initial linkage block is broken up by crossover at the meiosis. His approach is effective in analyzing the behaviour of linked genes in the course of transmission of chromosomes from generation to generation in different mating systems. The number of loci concerned does not restrict the analysis. He indicated the average and variance of the linkage block length held intact through generations in selfing, backcrossing and intermating.

The author investigated by applying the Monte Carlo simulation method how the parental linkage block on a pair of homologous chromosomes is broken up by crossovers in each selfed generation and is transmitted to the next generation for a varying number of crossovers per chromosome. The simulation model was set up so as to conform with actual features of crossover occurring at the meiosis as closely as possible in the light of cytological evidences. Based on these results, problems relevant to the fixation of a single pair of chromosomes and whole genome and the distribution of the number of linkage blocks finally obtained were discussed. Studies also aimed at determining how the parental chromosomes are recombined when anther culture is employed in plant breeding as a technique for obtaining complete homozygotes in a very early generation.

Simulation Procedures

A self-pollinated diploid crop species with somatic chromosome number $2n$ will be considered. Assuming that n pairs of chromosomes are all metacentric and each arm of the chromosomes is composed of 100 segments, the crossover point on a chromatid should fall between a segment and the adjacent one.

The change of the parental linkage blocks on a single pair of homologous chromosomes was investigated by applying the Monte Carlo simulation method. Simulation started in F_1 where one of the homologues is derived from the male parent and the other from the female. A digit 3 was assigned to all the segments of the chromosome from the male and 5 to those from the female. These digits were selected simply for the convenience of visualization of the chromosomes on a computer display. According to cytological investigations, crossover takes place between non-sister chromatids of paired homologues at the meiosis, with only one chromatid being involved in each crossover. Pseudorandom number was generated in a computer to choose the chromatid to participate in a crossover for the two chromosomes separately, and to determine the position along the chromosome of a crossover. Crossover between sister chromatids was neglected. The digit in all the segments distal to the crossover point on the selected chromatid was replaced by the digit on the counterpart segment of the partner chromatid. Occurrence of crossovers was repeated for a given number. Then one out of the four chromatids derived from a mother cell was chosen arbitrarily on the male and female

side separately to yield a chromosome which was to be contained in the gamete taking part in fertilization. The other three chromatids were discarded. A copy of each chromatid derived from the male or female gamete was made and added to the original to form sister chromatids. The chromosome was then united to produce a zygote or plant in the next generation i.e. F_2 . The procedures were similarly performed in every selfed generation until the digit of every segment on an entire chromosome became identical with the digit of the counterpart segment, in other words, complete homozygosity was attained for the pair of chromosomes. It was assumed that the multiplication of plants in each generation occurred through the single seed descent (SSD) method. No selection of genotypes and no mutation were assumed. The proportion of heterozygous length, the length of each linkage block and the position of the break point in each generation were printed out. The number of generations required for fixation and the number of breakups realized in the initial linkage block in the final generation were also recorded.

Six situations where the number of crossovers per chromosome (NCR) was constant throughout the generations (NCR=1, 2, 3, 4, 6 and 10) and 12 situations where NCR varied with the generations, for instance, 1 for F_1 and 4 in later generations were simulated in 100 runs and 10 replications for the former and 3 for the latter, respectively.

It was assumed that in a particular generation the number of crossovers does not follow the Poisson distribution but is fixed (see Discussion). No chromatid interference and independent distribution for the position of crossovers within the arm of a chromosome were assumed. The distribution of the crossovers between the arms of a chromosome was however non-random. It was assumed that when NCR is an even number $2m$, m crossovers occur on both arms and that when it is an odd number $2m+1$ then m crossovers occur arbitrarily on one of the two arms and $m+1$ on the other.

A programme written in Basic was developed for the simulation and transferred to MS-DOS on a micro-computer PC 9801 vm. Pseudorandom numbers were generated by using a function RND and the following arbitrary chosen numbers were given as the initial values by a statement RANDOMIZE : 25581, 17456, 14851, 24871, 30478, 11895, 15786, 21021, 11597 and 20489. Simulation runs for changes in linkage blocks from F_1 to fixation in 100 individuals were performed using random numbers consecutively generated with the same initial value. A period of $2^{18}=262144$ was achieved for the pseudorandom sequence irrespective of the initial value used. Time required for generating 10,000 random numbers was 6.9 sec.

Since the map distance x in terms of Morgan units between two loci is defined as the average number of crossovers per chromatid (OWEN 1950), NCR mentioned in the present report is equal to the double of the genetic map distance i.e. $NCR=2x$. For instance, if NCR was set at 4, the length of the chromosome corresponded to 200 centimorgans. Since it was assumed that each chromosome has 200 segments and that the crossover occurs in the links between two adjacent segments, it follows that two crossovers with a distance of less than $NCR/4$ centimorgans cannot be discriminated in the present simulation. This results in a slight underestimation of the number of breakups, especially when NCR is large, but the deviation is only 4 percent at most.

Results

Fixation of genes : In this report the term fixation refers to the attainment of homozygosity of all the genes on a single pair of chromosomes or all the pairs of chromosomes involved in the genome. The generation when fixation is attained will be called hereafter fixation generation for simplicity. Fixation generation for a single pair of chromosomes was first examined for six cases where NCR is 1, 2, 3, 4, 6 or 10. The distribution for the fixation generation obtained from a total of 1000 runs, as shown in Fig.1, was asymmetrical, tailing towards larger values for all the cases examined. When NCR was small a large proportion of the chromosomes became fixed in very early generations. For instance, when $NCR=1$, 12.6% of all the chromosomes were fixed in F_2 and 23.6% in F_3 . The average gradually increased as NCR became larger, but the increase was not conspicuous. Tenfold increase in NCR from 1 to 10 delayed fixation by only 3 generations. When the average was plotted against the logarithm of NCR, a linear relationship was recognized (Fig.2). The maximum among the 1000 runs was in the range from 14 ($NCR=1,2$) to 20 ($NCR=10$), which was about three times the corresponding average. The maximum obtained here naturally depends upon the total number of simulation runs performed and the ratio of maximum to average will be lower or higher according to whether the number of runs is less or more than 1000. The frequency of heterozygotes at a particular locus among all the plants in a population or the total length of heterozygous segments of chromosomes averaged over all the individuals is expected to be halved in every selfed generation irrespective of the linked or unlinked relation of the loci. If, however, the length of the heterozygous segments was averaged over only unfixed chromosomes, clear differences were noticed with different NCR (Table 1). The smaller the NCR, the lower the frequency of the chromosomes which still remained heterozygous, as described above. But the length of heterozygous segments in the heterozygotes was larger. In addition, the variation between

Fig.1. Frequency distribution of fixation generation for different numbers of crossovers per generation (NCR). Total frequency is 1000. A vertical bar stands for maximum limit of range.

Fig.2. Relationship between the number of crossovers per generation and the fixation generation averaged over 1000 simulation runs

Table 1. Degree of heterozygosity of an entire chromosome averaged over unfixed chromosomes as a function of selfed generations

No. Crossover	Generation										
	1	2	3	4	5	6	7	8	9	10	11
1	1.000	.572	.391	.314	.249	.215	.177	.139	.114	.108	.097
2	1.000	.519	.302	.204	.144	.110	.092	.077	.079	.072	.097
3	1.000	.505	.281	.175	.123	.091	.072	.064	.065	.069	.061
4	1.000	.504	.268	.160	.107	.079	.066	.057	.047	.044	.048
6	1.000	.500	.256	.145	.094	.069	.048	.039	.030	.027	.021
10	1.000	.500	.251	.133	.077	.050	.036	.028	.024	.026	.021
Ind.	1.000	.500	.250	.125	.062	.031	.015	.007	.003	.001	.000

¹⁾ Ind. = Independent segregation of a large number of loci

chromosomes in heterozygous length differed with the value of NCR. The variation was much larger for smaller values of NCR. When NCR=1, for instance, chromosomes heterozygous in a few percent of the segments to over the entire length were recognized in F_5 , while in case of NCR=10 heterozygous length never exceeded 35% in any chromosomes (Fig. 3).

Cumulative frequency of complete homozygous chromosomes as a function of generations

Fig. 3. Proportion of heterozygous length of chromosome in F_5 for different numbers of crossovers (NCR).

Fig. 4a, b. Cumulative frequency of complete homozygotes as a function of generation. (a) present results, (b) curves of Jones

of selfing is shown in Figs. 4 a and b, along with the curves of JONES (1918) for independent factors. By comparing the generation in which 50% of the chromosomes or individuals reach complete homozygosity between the two figures, it was noticed that a number of 1, 2, 3, 4, 6 and 10 crossovers per chromosome was, roughly speaking, equivalent to a number of 3.5, 8.0, 11.1, 14.2, 20.3 and 33.2 unlinked genes in terms of the rate of progress to fixation.

The fixation of a whole genome in which n pairs of chromosomes are involved will be considered. The cumulative frequency of individuals with a complete homozygous genome can be expressed as the product of all the cumulative frequencies for each pair of chromosomes. Setting NCR as a constant for all the pairs of chromosomes and equal to 2, the cumulative frequency of complete homozygous individuals was calculated for $n=2, 7, 12$ and 21, as shown in Fig. 5. The latter three numbers were chosen because they are equal to the half number of somatic chromosomes of the three main self-fertilizing cereals *i.e.* barley, rice and wheat, respectively. The results obtained in the present simulation can be applied to common wheat, too, since, as is well known, the species usually exhibits regular bivalent formations and diploid-like segregation of the chromosomes at the meiosis. When only a single pair of chromosomes was concerned, the frequency rose from F_2 onwards, began to taper in F_7 and exceeded 95% in F_9 . As n increased, the curve moved towards later generations with its shape being almost

Fig. 5. Cumulative frequency of complete homozygotes as a function of generation for different numbers of chromosomes in the genome. The number of crossovers per generation was set at 2. n is the half number of somatic chromosomes.

Fig. 6. Cumulative frequency of complete homozygotes as a function of generation for different numbers of crossovers per generation. The half number(n) of somatic chromosomes is 7 (top) or 12 (bottom).

unaltered. When $n=7$, the frequency did not take off until F_6 and the frequency reached 95% in F_{12} . With $n=21$, the frequency began to rise in F_8 and reached 95% in F_{14} . Although the shift of the curve with the increase in n from 7 to 21 was only 2 generations, the difference in the frequencies in a particular generation was large during the generations from F_7 to F_{10} , since the curve was very steep for the first three generations after taking-off. The cumulative frequency curves of complete homozygotes for different NCR with a fixed $n=7$ or 12 are shown in Fig. 6. The curves shifted towards later generations as NCR increased with little alteration in their shapes. The delay of the increase of the complete homozygotes in accordance with the change in n from 7 to 12 was almost constant and equal to about one generation irrespective of the NCR value.

Breakup of linkage blocks : Next, studies were carried out to determine how many the parental linkage blocks on a chromosome are broken until homozygosity is attained over the entire length of the chromosome. The distributions for the number of breakups for different NCR, as shown in Fig. 7, were asymmetrical and tailed towards larger values. As NCR increased, the range of distributions widened. The maximum number of breakups among 1000 runs ranged from 5 (NCR=1) to 17 (NCR=10). When NCR was small, the probability for the parental linkage blocks to be transmitted intact up to the fixation generation was high and reached 45% when NCR=1. The average number of breakups increased almost in proportion to the value of NCR (Fig. 8). The ratio of the number of breakups to NCR was nearly constant and in the range from 0.73 (NCR=10) to 0.98 (NCR=2).

Fig. 7. Frequency distribution of the number of breakups of the initial linkage block for different numbers of crossovers per generation. Total frequency is 1000. A vertical line stands for the maximum limit of the range.

Fig. 8. Relationship between the number of crossovers per generation and the number of breakups of initial linkage block in the fixation generation averaged over 1000 simulation runs.

Fig. 9. Illustrative examples for the lengths of linkage blocks in the fixation generation for different numbers of crossovers per generation (NCR).

generation was thus even smaller than the number of crossovers occurring on a chromosome in every generation. As an illustration, some patterns of linkage blocks observed on a chromosome in the fixation generation for different NCR are shown in Fig. 9.

Since the number of breakups of initial linkage blocks and fixation generation under a given NCR vary with the chromosomes, the relationship between the two was examined. There was a positive and significant correlation at 1 percent level for all the NCR. The correlation

table for NCR=2 is shown in Table 2. The correlation was comparatively closer for smaller NCR. For crop improvement, highly recombined plants may be required as potential candidates for the selection of desired genotypes. Thus the maximum rather than average number of breakups is important in considering the efficiency of breeding systems in terms of recombination of the parental genotypes. In this connection investigations were carried out to determine how the fixation generation changed when 1 percent of the individuals (10 runs) with the highest number of breakups was selected from 1000 individuals in which each of the six cases of NCR had been simulated (Table 3). Obviously, the average number of breakups remarkably increased in the selected individuals, resulting in values being several times those of the original value. On the other hand, the number of generations to fixation was not conspicuously increased and the average was substantially constant *i.e.* around F_9 irrespective of NCR.

Table 2. Correlation table between fixation generation and the number of breakups of initial linkage block in the case of NCR=2 ($r=0.492^{**}$)

Fixation generation	No. breakups of linkage block									Total
	0	1	2	3	4	5	6	7	8	
1										0
2	37									37
3	27	76	32	1						136
4	19	88	84	21	3					215
5	11	44	72	37	11	1	2			178
6	10	39	52	20	20	9	1			151
7	4	19	34	29	17	10	1			114
8	3	9	18	9	13	13	1	1	1	68
9	1	8	12	14	8	5	4			52
10		4	8	5	2	2	1			22
11		2	3	7	3	1	1			17
12	1	2		1	1		1			6
13				1						1
14			1		2					3
Total	113	291	316	145	80	41	12	1	1	1000

Table 3. Average number of breakups of initial linkage block and fixation generation when 1% of the chromosomes with the highest number of breakups was selected

No. crossover	No. breakups		Fixation generation	
	Range	Mean	Range	Mean
1	4—6	5.0	6—13	9.0
2	7—9	7.3	5—12	8.2
3	8—10	8.6	6—12	7.9
4	9—12	10.3	8—14	10.6
6	13—15	13.3	7—12	9.5
10	16—18	16.8	7—13	9.0

Table 4. Number of breakups of the initial linkage block when the number of crossovers was not constant between the generations

No. crossovers	No. breakups														Ratio ¹⁾
F ₁ F ₂ F ₃ F ₄ F ₅ F ₆	0	1	2	3	4	5	6	7	8	9	10	11	Total	Average	
1, 1, 1, 1, 1, 1	351	426	156	58	8	1							1000	.95	1.00
4, 1, 1, 1, 1, 1	62	224	315	237	118	26	15	3					300	2.27	2.38
1, 4, 1, 1, 1, 1	70	87	63	48	25	4	3						300	1.65	1.74
1, 1, 4, 1, 1, 1	85	110	39	52	8	2	3	1					300	1.37	1.44
1, 1, 1, 4, 1, 1	93	130	46	24	6	1							300	1.07	1.13
1, 1, 1, 1, 4, 1	106	130	43	18	3								300	0.94	0.99
2, 1, 1, 1, 1, 1	48	119	85	35	8	4	1						300	1.51	1.59
3, 1, 1, 1, 1, 1	42	79	96	67	13	3							300	1.80	1.89
2, 2, 2, 2, 2, 2	113	291	316	145	80	41	12	1	1				1000	1.97	1.00
4, 2, 2, 2, 2, 2	13	58	84	68	44	21	7	3	2				300	2.63	1.34
6, 2, 2, 2, 2, 2	3	20	62	68	76	44	16	6	5				300	3.50	1.78
3, 3, 3, 3, 3, 3	70	194	235	220	137	87	38	14	4	1			1000	2.67	1.00
4, 3, 3, 3, 3, 3	13	49	69	61	55	29	14	9	1				300	2.97	1.11
6, 3, 3, 3, 3, 3	4	18	48	57	72	50	23	20	7	1			300	3.89	1.46
4, 4, 4, 4, 4, 4	41	127	181	196	199	113	75	47	12	6	2	1	1000	3.40	1.00
6, 4, 4, 4, 4, 4	4	13	31	61	69	54	33	18	14	2			300	4.23	1.24

¹⁾ Ratio of the average number of breakups for varying vs. constant number of crossovers

Since the increase in NCR was effective in breaking the parental linkage block, studies were carried out to determine whether its effectiveness can be expected even when the increase in NCR was performed in a particular single generation, as shown in Table 4. When NCR in F₁ was increased from 1 to 4 without changing NCR in the later generations, the average number of breakups increased from 0.95 to 2.27, which was greater than the average expected when NCR was 2 throughout the generations. The widening of the distributions for the number of breakups with the change in NCR was also observed. The later the generation at which the change in NCR was performed, the smaller was its effect. The average number of breakups expected when NCR in F₁ was changed from z1 to z2 was nearly equal to the mean of the average number for the two cases where NCR was z1 or z2 for every generation.

Finally, the efficiency of anther culture as a technique for obtaining complete homozygo-

Table 5. Number of breakups of the initial linkage block in F_1 gamete as compared with that obtained in the fixation generation in ordinary selfed generations

No. crossovers	No. breakups						Ratio (B/A)
	F ₁ gamete			Fixation generation			
	Mean (A)	Range	Variance ¹⁾	Mean (B)	Range	Variance ¹⁾	
1	0.52	0—1	.250	0.95	0— 5	.825	1.83
2	0.96	0—2	.456	1.97	0— 8	1.830	2.05
3	1.36	0—3	.752	2.67	0— 9	2.724	1.96
4	1.73	0—4	1.060	3.40	0—11	3.659	1.97
6	2.32	0—5	1.569	4.69	0—14	5.156	2.02
10	3.78	0—7	2.065	7.31	1—17	8.372	1.93

¹⁾ Variance of individual runs

tes in a very early generation was compared with the ordinary fixation in selfed generations in terms of breakup of linkage blocks (Table 5). The average was nearly equal to half of that obtained in ordinary fixation while the maximum was much reduced. Even when NCR=10, the maximum obtained by the anther culture method was only 7, which was smaller than the maximum for NCR=2 by the ordinary method.

Discussion

In simulation studies on the recombination and fixation of genes, the reliability of the results obtained depends upon how the model adopted in the simulation procedures is realistic in the light of cytological evidences.

It has often been assumed in theoretical studies on crossover that the distribution of the number of crossovers on a chromosome follows the Poisson distribution (OWEN 1950, HANSON 1959 a). Cytological work, however, indicates that this assumption is not verified in normal diploid species. The frequency of crossovers is deduced from that of chiasmata cytologically observed. If the distribution were of the Poissonian type, the variance would be equal to the mean. But there are several reports which indicate that the actual distribution for the number of chiasmata per chromosome is much narrower than expected from the Poisson distribution (MAEDA 1930, SAX 1934, MATHER 1934). Furthermore, JONES (1967) showed that an abnormal genotype derived from an interspecific cross of *Secale* species has an independent distribution of chiasmata between cells, between bivalents and within bivalents with the number of chiasmata per chromosome fitting to Poisson distributions. In a normal genotype derived as sibs from the same cross the distribution was again much narrower. JONES suggested that a genetic control mechanism is involved in the maintenance of efficient chiasma conditions. Another point which shows a divergence of the actual distribution of chiasmata from the Poisson distribution is the rarity of bivalents without chiasmata. The probability for a bivalent not to possess a chiasma on it would not be negligible if the distribution were random, particularly when the chromosome is short and the average number of chiasmata is small. Yet cytological observations clearly indicate that at least one chiasma is always detected in a bivalent in normal plants. Without a chiasma a pair of homologous chromosomes can not be

kept together until the metaphase and the normal orientation of the chromosomes is impaired. Non-randomness in chiasma distribution is recognized also between arms of the same chromosome. According to CALLEN and MONTALENTI (1947), in *Theobaldia*, bivalents with an equal number of chiasmata on each arm show a strikingly higher frequency than those with an unequal distribution of chiasmata between the arms. With these cytological evidences in mind it was assumed in the present simulation model that the number of crossovers per chromosome was fixed, with their distribution between arms being as equal as possible. There are several reports indicating that a chiasma tends to be localized on particular parts of the chromosome (HENDERSON 1963, SOUTHERN 1967), but the patterns of distribution may depend upon the species examined and few data relevant to the problem are available in crop plants. It was thus thought that it would be preferable to determine the points of crossover along the length of a chromosome at random.

Self-fertilization leads rapidly to homozygosis. The frequency of heterozygotes at a particular locus averaged over the whole population is halved at each generation of selfing regardless of whether the genes are linked to each other. The linkage of the genes on a chromosome affects the speed of attainment of fixation of all the genes on a chromosome and the variations in the total length of heterozygous segments in the chromosomes. If no crossover were to occur on a chromosome at all without disorder of normal pairing and segregation of the bivalents in meiosis, the chromosome would behave as a single locus in the fixation and recombination of genes. The present results (Fig. 4a, b) indicated that one crossover per chromosome in every generation has such an effect that it would divide one chromosome into 3.5 to 4.0 independent factors in terms of the progress towards fixation. It is not the number of genes or loci involved in a chromosome but the number of crossovers on a chromosome that determines the speed in attainment of fixation of all the genes on the chromosome. The number of generations needed for the fixation of the genes involved in a whole genome depends upon the number of pairs of homologues in addition to the number of crossovers per chromosome. More simply, it depends on the total number of crossovers in all the bivalents, since, as described above, a chromosome behaves like an independent factor with its number which is proportional to the number of crossovers per chromosome. Another feature of the linked genes is the presence of wide variations in the total length of heterozygous segments in a chromosome. If N genes on a chromosome were all genetically independent, then, assuming that the frequency of heterozygous loci is h , the variance of the frequency between individuals would be $h(1-h)/N$ which is extremely small if N is the total number of genes on a chromosome. In other words, plants are more uniformly heterozygous in the absence of linkages. The presence of linkages strikingly enhances the variation in frequency of heterozygous loci on a chromosome, especially when the number of crossovers per chromosome is small. For instance, when $NCR=1$, a large proportion of the unfixed chromosomes are expected to have a high frequency of heterozygous loci in a generation as late as F_5 in spite of the high frequency of fixed chromosomes. Thus, if final individual selection is performed in too early generations in practical breeding procedures, the chance of obtaining highly heterozygous plants is much greater than might

be generally considered. The heterozygosity remaining in the selected plants induces undesirable intravarietal variations and may result in degradation of the genotype of the released variety. The fixation or absence of fixation of all the genes rather than the average frequency of homozygous loci of the plants to be selected is of essential importance in considering the problem of fixation in hybridization breeding.

The number of breakups of the parental linkage blocks finally obtained in a fixation generation is a good measure of the efficiency of breeding procedures in the recombination of the parental genes. The number of breakups, it has been found, was proportional to the number of crossovers per chromosome in a generation. The ratio of the former to the latter was not large, being smaller than 1. This phenomenon may be ascribed to the fact that a crossover occurring within a region of homozygous parts of a chromosome is not effective in changing the combination of parental genes and that a crossover is also ineffective unless its formation takes place in the chromatid which is selected by chance to be transmitted into the next generation.

To obtain plants with as high a recombination level as possible, selection of individuals in later generations and artificial increase of crossover frequency particularly in early generations are recommended. An advantageous feature of growing plants without selection until later generations has very often been discussed by many researchers and does not need to be mentioned here. As an opposite extreme, regeneration of plants from cultured anthers along with doubling of chromosomes is sometimes recommended as a technique for obtaining complete homozygotes in very early generations. In that case, as crossovers occurring in F_1 are the sole source of recombination, plants with a high level of recombination are unlikely to be formed. The average number of breakups was half of that obtained in ordinary fixation. It is worth noting however that the maximum number of breakups was much smaller by the application of the anther culture method which indicates the loss of highly recombined plants that could be obtained otherwise. The method is recommended only in situations where recombination of genes independent or very loosely linked to each other is sufficient to obtain the desired plants.

Temperature during the meiotic stages (NOLTE *et al.* 1969, LU 1974, DEHAYES 1978), irradiation with gamma-rays (LAWRENCE 1961, OMURA and YAMAGUCHI 1971) and $^3\text{H}\beta$ -rays (SINGH *et al.* 1974), treatment with agents such as mitomycin (SUZUKI 1965), actinomycin D (SINHA and HELGASON 1969) and FUdR (WOLFF and SERRES 1967) are known to be more or less effective in increasing the frequency of chiasma or recombination. Genetic control over chiasma frequency has also been reported (REES and AHMAD 1963, CROWLEY 1969, NOLTE *et al.* 1969, GALE and REES 1970). As these data indicate, experimental increase of crossover by artificial means is possible. However, a magnitude of the increase sufficiently high for the present purpose has not yet been reported, as far as the author is aware. More effective methods remain to be developed.

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自殖世代における遺伝的固定と連鎖切断

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自殖性2倍体作物の2純系間交配において, 染色体当りの乗換数を種々に変えた場合に, 交配後の自殖世代で染色体または全ゲノムの遺伝子が何世代目でホモ接合になる(完全に固定する)か, および両親の原連鎖ブロックが固定世代までに幾つのブロックに切断されるかをモンテカルロ・シミュレーションによって調べた。染色体あたり乗換数を毎世代一定(1, 2, 3, 4, 6, 10), または, 変化させた計10通りの条件に対して, 毎世代の雌雄減数分裂における乗換の位置, 腕間分布, 関与染色分体の選択を一樣擬似乱数で決めて自殖世代を模擬

し、一対の相同染色体が固定するまで世代を繰り返した。染色体あたり乗換数は Poisson 分布ではなく一定とした。また、同じ染色体の腕間の乗換数は同数になるように定めた。各条件につき 1000 (世代間乗換数一定) または 300 試行 (乗換数不定) とした。

(結果) (1) 乗換数が増すにともない、1本の染色体の全遺伝子が固定するまでの世代数は増加したが、増加率は比較的lowく、1から10へ乗換数が増しても平均固定世代は4.25から7.32と、3代しか遅れなかった。固定世代は乗換数の対数と直線関係にあった。(2) 乗換数が1, 2, 3, 4, 6, 10の場合の一対の染色体が固定する頻度の世代に対する累積曲線は、JONESの独立因子の場合の3.5, 8.0, 11.1, 14.2, 20.3, 33.2にそれぞれ近似的に相当していた。言換えると、完全固定に至る速度からみて、染色体当りの乗換は1回につき、染色体を3.3から4.0個の独立因子に分割することに相当していた。(3) ゲノムの全染色体が固定する世代は、染色体当りの乗換数だけでなく、染色体数 $2n$ に依存した。乗換数が2の場合、95%が完全固定するのは、一対の染色体では F_9 であったが、 $2n=14$ では F_{12} 、 $2n=24$ では F_{18} であった。乗換数の増加による平均固定世代の遅れは、染色体数に関係なく同程度であった。(4) 固定世代に最終的に得られる連鎖切断数は、毎代の乗換数よりも少なく、その比は0.73から0.98の範囲にあった。その分布は、非対称で値が大きいほうに尾を引いた。平均値は乗換数とともにほぼ比例して増加し、最大値は5から17の間にあった。(5) 乗換数を一定にした1000回の試行の間で、固定世代と連鎖切断数とは正の有意な相関(.341から.557)を示し、乗換数が小さいほど相関は高い傾向があった。(6) 連鎖切断数の高いほうから1%の染色体対を選抜すると、連鎖切断数の平均は当然高まり5-17に達するが、固定世代は乗換数に関係なく平均9前後であった。(7) 乗換数を特定の一代だけ増加させた場合、その増加が初期世代に行われるほど、連鎖切断数の増加に有効であった。 F_1 での効果は、全世代の乗換数を同数だけ増加させた場合の効果の半分に達した。(8) 薬培養において F_1 配偶子からの個体再分化と染色体の倍化を行ったときの連鎖切断数は、平均的には通常の固定世代の場合の半分に達したが、最大値は著しく低くなり、例えば、薬培養での乗換数6の場合の最大値は固定世代での乗換数1の場合の最大値程度でしかなかった。