

Chromosomal Divergence in Three Natural Populations of Corchorus Olitorius Linn.

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I. INTRODUCTION

The role of chromosomal aberrations in species change has been a subject of wide-ranging investigations for some time. Not a few workers consider the restructuring of chromosomes a fundamental mechanism leading to genetic variability which may eventually bring about the divergence of populations of a species. Reports of spontaneous alterations in morphology of chromosomes of many species in the various regions of the world indicate the universal and dynamic nature of population change.

A cytological study of the genus *Clarkia*¹² showed that populations of several species had chromosomal differences as evidenced by the different types of restructured chromosomes in them. Chromosome repatterning could bring together adaptive gene complexes formerly dispersed on two or more chromosomes³ thereby initiating genetic variation in a species. Rather than point mutations, rearrangements in the genetic material could be an effective means of achieving new patterns of genetic regulation²³ in a population. Chromosomal restructuring could result in reproductive isolation² of a population wherein chromosomally-altered plants develop a limited capacity to form hybrid seeds thereby serving as an isolating mechanism against possible genetic "contamination"¹⁹. In a related view, a structural chromosomal change in a population could cause adaptive uniformity resulting from a decreased rate of recombination, adaptive linkage, new adaptive gene frequencies and new adaptive mutant alleles²². In *Secale cereale*, a close relationship was found between repatterned chromosomes and changes in the breeding system where new linkage combinations possessed some adaptive value for colonizing species¹⁵.

Of general interest is the report⁵ that many of the so-called single locus mutations frequently detected in natural populations are actually chromosomal deletions. The close relationship between chromosome restructuring and gross morphology of individuals in a population has been aptly stressed in the statement that "Morphological diversity is paralleled only by karyological diversity"¹⁶. Indeed, studies on chromosome repatterning have provided evidences for phylogenetic relationships among plant and animal groups and have served to check some speculative trends in the evolution of certain species.⁷

The plant, *Corchorus olitorius* Linn. (Tiliaceae), was reportedly introduced into the Philippines from India probably in pre-historic times and has since become widely distributed in the different areas of the archipelago. Very few studies, if at

all, appear to have been done on the nature of chromosomal differences among populations of introduced or migrant species as such. How much have the populations of such species diverged chromosomally from each other in their new habitats and from races indigenous to the place of origin is a subject of great biological interest. This work is dedicated toward nourishing that interest and is an attempt to study some aspects of the chromosomal repatterning process in populations of the species. This paper takes into account only the types and frequencies of different meiotic configurations in each population.

II. MATERIALS AND METHODS

Young flower buds of *Corchorus olitorius* Linn. ($N = 7$) were collected at random from large populations of the plant in Bantay, Ilocos Sur; San Miguel, Tarlac; and Janiuay, Iloilo. The plant materials were fixed in Farmer's fluid (3:1 parts ethanol and acetic acid) and stored in 70 percent ethanol at low temperature until at least a day prior to microscopic study. The cytological specimens were prepared employing the p.m.c. smear technique using aceto-carmin stain. Meioocytes at different stages of meiosis I were examined for chromosomal configurations due to the repatterning of chromosomes.

For the purpose of this work, the chromosomal configurations were grouped into three types, viz., interchange complex, anaphase I variants and altered chiasma associations at metaphase I. Although the mitotic chromosomes of *C. olitorius* are rather small, the meiotic⁶ chromosomes are fairly large and suitable for analysis due mainly to the small gametophytic number of only seven bivalents. Validity of the cytological data from the Ilocos Sur population is evidenced by the report¹⁰ that intensive harvesting of natural stands does not produce any chromosomal or genetic effect on the population.

III. RESULTS AND DISCUSSION

Out of a collection of 1,116 plants from the three populations, only 45 plants or 4.03 percent gave evidences of repatterned chromosomes. The Iloilo population gave the highest frequency of chromosomally-altered plants of 2.69 percent while the Ilocos Sur and Tarlac population yielded only 0.63 percent and 0.72 percent, respectively. The higher frequency of aberrant plants in the Iloilo populations than the other groups may be due to the high percentage of interchange plants with the alternate orientation. It is generally known that such translocation heterozygotes are capable of transmitting their

interchange complexes to a large portion of their progeny⁶. Whether through self-fertilization or testcross involving individuals with the viable type of reciprocal translocation, approximately 50-75 percent of their progeny inherit the interchange complex.

Based on the random plant samples, the three populations also differed in the predominant type of meiotic configurations. The Ilocos Sur population was essentially an "anaphase I variant group" with approximately 66.15 percent of the total aberrant meiocytes bearing dicentric chromatic fragments. The Iloilo population was predominantly an "interchange complex group" with approximately 84.97 percent of the total variant meiocytes bearing ring, zigzag or chain quadrivalents or only bivalents. The Tarlac population was mainly an "altered chiasma association group" with 68.75 percent of the total variant meiocytes giving slightly more chiasma associations than the normal number of 9.00 chiasmata per cell. The predominant type of aberrant meiotic configuration in a group is possibly related largely to the extent of its distribution in the population which in turn may be brought about mainly by the type of restructuring process involved as well as the viability of the cell or cells bearing the altered chromosomes, number of generations the repatterned chromosome has existed in the population and transmission of the repatterned chromosome.

An analysis of the meiotic data was made to determine some features of the chromosomal configurations in each population. Regarding chromosomal interchanges, the Ilocos Sur population gave mainly the alternate type of orientation with about 40.91 per cent of the variant meiocytes bearing the zigzag configuration at metaphase I. This indicates a high rate of gametic fertility and superior chances for survival. The high frequency of chain quadrivalents and only bivalents at metaphase I shows that relatively short chromosomal segments were probably involved in the restructuring process of such chromosomes. In the Iloilo population approximately 51.83 percent of the variant meiocytes gave ring quadrivalents which presumably results from the adjacent type of interchange orientation indicating that relatively long chromosomal segments were possibly involved in the re-patterning process. The Tarlac population gave a similar interchange pattern as the Ilocos Sur population. The cytological significance of different types of interchange complexes in a population lies in the possibility that more than two pairs of repatterned chromosomes in the genome are involved. There is no doubt that the existence of many restructured chromosomes in a population could increase the rate of genetic divergence of a group from other populations of a species granting that such chromosomal changes are not lethal combinations.

In a survey of chromosomal aberration in plants⁶ many species were found to carry interchanges as a typical component of their genetic systems and in *Clarkia angulata*, most of the structural changes were due to reciprocal translocations.¹⁴ Some populations of *Oenothera deltoides* and *O. avita* were reported to have higher frequencies of translocation heterozygotes than other groups of the same species¹¹. A cytological examination of natural populations of *Calycadena pauciflora* (Asteraceae) showed that a few individuals have reciprocal translocation⁷. In *Collinsia heterophylla* (Scrophulariaceae), the interchanges exhibited the alternate type of orientation giving zigzag and chain quadrivalents at metaphase I²⁰. Compared to heterozygotes, interchange homozygotes in the same population were found to have larger average and maximum biomass values⁴.

The anaphase configurations consisted per cell of 2 dicentric bridges and 4 acentric fragments, 1 dicentric bridge and 2 fragments, 1 dicentric bridge and 0 fragments, and 0 bridge and 1 fragment. In the Ilocos Sur population, approximately 81.39 percent of the aberrant meiocytes in anaphase I has a dicentric bridge with or without an acentric fragment and in the Iloilo population, about 55.18 percent of the cells gave these configurations. The variant meiocytes in the Tarlac population were too few to be validly taken into account. The anaphase I variant configurations probably had their origins from structural changes of the A-type, as compared to the S-type¹⁷, as evidenced by the microscopically-visible chromosome associations. As such, their formation most probably resulted from the mechanical separation of restructured chromosomes as the homologues migrate toward the spindle poles. The frequency of cells bearing an anaphase I variant configuration is probably related largely to the number of repatterned chromosomes, type of restructuring process, site of breakage and transmission of the repatterned chromosome.

In Indian barley¹⁸, the occurrence of a dicentric bridge at anaphase I did not affect the percentage of aborted pollen grains compared to the standard strains. Anaphase bridges and acentric fragments at meiosis I are believed to have resulted from inverted duplications or from spontaneous chromatic breakage and re-union⁸. In some populations of several species in the genus *Clarkia*, most of the anaphase I configurations were found due to inversion.¹³ It is quite probable that the dicentric bridges in anaphase I in *C. olitorius* may have similarly originated from heterozygous paracentric inversions and the acentric fragments may have resulted from closely adjacent breakages along the chromatic bridge.

The three populations of *C. olitorius* also differed in number of chiasma associations resulting in a slight change in chiasma frequency which is the mean number of chiasmata per cell at metaphase I²¹. Some investigators consider chiasma frequency as a general cytological measure for estimating the relative length of the genome⁹ while others believe that it reflects the

amount of genetic recombinations over a wide range of organisms³. These assumptions are invariably based on the idea that chiasma frequency remains more or less constant in the normal meiocytes of an individual and individuals of the same species¹.

The Tarlac population gave a slightly higher number of chiasma associations giving a chiasma frequency of 10.04 compared to the other groups. Although the difference is not statistically marked, the slight increase is obviously due to the meiocytes bearing more ring bivalents than in normal germ cells. All the 44 variant meiocytes of this population gave 4 rod and 3 ring bivalents or 3 rod and 4 ring bivalents as compared to the normal chromosome complement of 5 rod and 2 ring bivalents. The more significant aspect of this slight increase in chiasma frequency lies in the possible restructuring of at least 1 or 2 rod bivalents into ring bivalents probably at some point or points in the long evolutionary past of the population. This may have been accomplished through an increase in the length of the unpaired arms of the rod bivalents through some kind or kinds of chromosome repatterning processes. Similar changes may have taken place in *Limnanthes floccosa* where the increase in chiasma frequency from a range of 8.50 - 9.00 to 10.20 was due to the greater number of ring-type bivalents than the normal genome, each ring bivalent with two localized or subterminal chiasmata resulting in greater genetic variability through recombination and, in fact, eventually led to the development of autogamy in the species¹.

SUMMARY AND CONCLUSION

It is evident from the foregoing data that some degree of chromosomal divergence have taken place in the three natural populations of *C. olitorius* Linn. (Tiliaceae) in the frequency of chromosomally-aberrant plants, predominant type of meiotic configuration, frequency of anaphase I variant meiocytes and chiasma associations.

The higher frequency of plants with repatterned chromosomes in the Iloilo population than in the other groups may be ascribed to the capacity of translocation heterozygotes to transmit their interchange complexes to a large portion of their progeny.

As the predominant type of meiotic configuration and frequency of anaphase I variant meiocytes depend to a large degree on the kind of restructuring processes involved, it is not unreasonable to assume that different types of chromosome repatterning occurred in the three populations.

The change in chiasma associations probably resulted from the repatterning of at least 1 or 2 rod bivalents into ring bivalents at some points in the remote past of the population.

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CHROMOSOMAL DIVERGENCE IN THE THREE NATURAL POPULATIONS OF *CORCHORUS OLITORIOUS* LINN. I MEIOTIC CONFIGURATIONS

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Discussant

The presence of chromosomal divergence in natural populations is an indication that although the genetic material is stable, it is capable of variation, making possible the formation of races, varieties and/or new species. Accumulated slight changes that occur from time to time in certain members of the population may lead to reproductive isolation. Various meiotic configurations are used as indications of the change in chromosome structure. Drastic changes in the chromosome like large deletions are lethal especially in homozygous condition and therefore are not efficient in producing variations which could be inherited. Changes resulting from reciprocal translocations and inversions may be passed on to progeny especially those in the homozygous condition because there is no change in the amount of genetic materials and normal pairing of homologous chromosome may take place during meiosis.

Translocation and inversion heterozygotes form various meiotic configurations as shown to us. Translocation heterozygotes could be fertile or sterile, depending upon the nature of segregation in anaphase I. Segregation in anaphase I could be adjacent-1, with homologous centromeres going to the same pole and adjacent-2 with non-homologous centromeres going to the same pole. Both adjacent-1 and adjacent-2 segregations lead to duplications and deficiencies at the same time which usually produce inviable gametes. Viable gametes are produced in alternate segregation, where the two normal chromosomes go to one pole and 2 chromosomes with a whole complement of genes. If the 3 types of orientations with reciprocal translocations go to the other pole producing gametes occurred at random, $2/3$ of gametes are expected to be inviable. However, in some plants the alternate segregation occurs with a much higher frequency as in this case. Probably there are factors governing the orientation of a ring of chromosomes on the metaphase plate. The more flexible the ring, the greater is the opportunity for maneuvering the chromosomes to give the alternate disjunction.

In translocation heterozygote, the chromosomes involved in a ring are not inherited independently, since two normal chromosomes are inherited as a group, as are the two translocated ones. What were before two independent linkage groups are now united into one, despite the fact that the chromosomes exist as independent entities. As the translocation complex increase in size, the number of independent linkage group decreases until a point

where all the genome may behave as one large linkage group. This will sharply reduce the component of variability due to independent assortment of chromosomes. Recombination of genes is confined to the pairing ends of each chromosome as shown in the slide. Recombination is not only reduced, but there is also an isolation of blocks of chromatin that allows for the accumulation of genetic differences upon which natural selection can act. One source of speciation is the establishment of characteristic linkage relationship. A change in linkage or in position of some genes due to translocation and inversion may lead to position effect. This is a phenotypic effect which is dependent upon a change in position on the chromosome of a gene or groups of genes.

Duplications could probably give rise to increase chiasma frequency. Duplications could be tolerated by the organism and could provide a feasible method for the acquisition of new genes, and hence new physiological functions. Two identical genes can divert through mutation. In well-adapted organisms, mutations in general, lead to a loss or impairment of function and are consequently likely to be selected against in a population because of their adaptive disadvantage. Should a mutated gene be present as a duplication along with the normally functioning gene, the possibility of its retention and continued mutation, possibly in new directions, become enhanced.

A change in karyotype may also lead to some ecological isolation as in the marine snail. In this organism, 18-chromosome snails below the low-tide level, the 13-chromosome snails form at high-tide level, and the intermediate-numbered individuals in the intervening tidal zone. It appears therefore that the different chromosomal races exhibit ecological preferences. The varying number and structure of genome is due to centric fusion where variable chromosome number can occur without apparent gain or loss of chromatic material. Two acrocentric chromosomes may fuse to form one metacentric or sub-metacentric chromosome. Dissociation on the other hand, can transform a metacentric chromosome into 2 acrocentrics. Centric fusion or dissociation takes place without impairment of centromeric function.

Please allow me to congratulate Dr. Joventino Soriano for his study in chromosome divergence. Chromosomes are microscopic structures that are quite difficult to study and unless one has patience, interest and above all a good background of cytogenetics, a paper like that of the speaker cannot be prepared.

Before I end, I would like to thank the Academy for inviting me as a discussant and Dr. Soriano for suggesting my name. I have a question for Dr. Soriano. In the title he put as number 1 meiotic configurations. I'm curious to know the next one to follow.

DR. SORIANO:

That is a very good question. May be the second part of this research, granting that I have the time would be to identify the

specific chromosomes involved in these changes. These species has 7 pairs of chromosomes and it will be very interesting really to determine which of the chromosomes in the Iloilo population are involved in this formation of ring and chain and zigzag and also which of the chromosomes in the Tarlac population gave these bridges and all these segments that we saw in the slides. Another important, may be third part which will be using segments in aberrations to determine where specific genes or factors of economic value are located in these chromosomes. And that would possibly add very much to our knowledge as a whole.

DR. CARMEN C. VELASQUEZ:

I would like to ask one question, in the expression of the cytogenetics, but cultural knowledge of genotype did you work on the phenotype?

DR. J. SORIANO:

So far this has been a work on cytology or chromosome. I think that should be the fourth study following this.