Chiasma Formation in a Heteromorphic Bivalent in *Belamcanda chinensis*, Leman

R. P. Roy and J. Saran

Botany Department, Patna University, Patna, India

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Introduction

As a result of spontaneous changes in chromosomal complements the shapes and sizes of these otherwise permanent structures undergo modifications. Therefore cytological investigations of specimens from natural populations furnish informations on the types of changes occurring in nature. The appearance of a heteromorphic pair of chromosomes in any organism is the result of spontaneous deletion in any one arm of chromosome, the other homologue remaining unchanged. They form heteromorphic bivalents at meiosis. This type of bivalent has been observed in many different plants and animals. Although the role of deletion in evolution has been very critically discussed by Dobzhansky (1951), Stebbins (1950), and in several recent text books (Swanson 1957); but the heteromorphic bivalent has not been used to a considerable extent in the understanding of the nature of chiasma formation. The nature of chiasma formation as enunciated by Janssens (1909, 1924) and elaborated by Darlington (1930, 1937) appeared to have been generally accepted by cytogenetists especially after the cytological demonstration of crossing over by Creighton and McClintock (1931) in maize and Stern (1931) in *Drosophila*. But in some recent research publications and text books, the general validity of the chiasma type theory has been questioned. The sources of evidence in favour of the partial chiasma type hypothesis were produced from multivalent configurations in structural heterozygotes, double interlocking, similar effects of temperature on the chiasma frequency and the percentage of crossing over etc. Evidences from all these sources are indirect ones and therefore, howsoever, convincing and logical they may be, they have been interpreted in a different way also. Thus double interlocking as studied by Mather (1933) was considered an excellent demonstration of partial chiasma type hypothesis. But Matsuura (1940) interprets even this evidence in a different way. Therefore more and more reliance has to be placed on the source of evidence which is considered as a direct one. Heteromorphic bivalent provides the source where the nature of chiasma formation can be studied directly, but this type of investigation has been made on a very limited number of organisms. Further if any marker gene is located on the unequal arm that may greatly strengthen the cytological observations.

In the present investigation, therefore, detailed studies of the hetero-

morphic bivalent which was found in a plant of *Belamcanda chinensis* growing in the garden amongst a group of 20 plants, has been carried out with a view to find out whether the chiasma formation in this bivalent supports or contradicts the partial chisma type hypothesis.

Materials and methods

The anthers from plants of *Belamcanda chinensis* were collected for general cytological studies, the anthers in this plant are quite long and therefore they were cut into pieces while putting them into fixative. They were directly put into 1% propiono carmine for 24 hours. Anther pieces were then taken out and squashed in 45% propionic acid. The slide was watched under the microscope till the extra stain from PMC was dissolved. The slide was then made permanent by the usual method. The routine method of fixing in acetic alcohol and then squashing did not give satisfactory results. While studying these slides from squash preparation of PMC of different plants, one particular plant showed a clear heteromorphic bivalent at meiosis. More squash preparations from this plant were then prepared to study the different stages of meiosis. Root tip squash was also made by pretreating the tip in saturated solution of para-dichloro-benzene for 2 hours, fixing in propionic alcohol (1 : 2) before staining and squashing in 1% propiono carmine. Microphotographs were taken from permanent slides.

Observations

The somatic chromosome number in this species is 32 (Fig. 1). The detailed cytological studies of this and other allied species is being published elsewhere. In this figure the heteromorphic pair is clearly seen at 5 o'clock.

Diplotene. Attempts were made to follow the chromosome pairing in the heteromorphic bivalent from the onset of chiasma formation, that is, early diplotene. In several PMCs good preparations of diplotene chromosomes could be observed. In one particular case where the heteromorphic bivalent with one chiasma in the unequal arm was quite separate from other bivalents; photograph has been taken (Fig. 2). In this particular case two chiasma in the equal arms and one in the unequal arms are clearly seen. The centromere point is also clearly visible. The two strand nature of the chromosome in the arm in which deletion has occurred is clearly seen just before the point where chiasma has been formed. Then on the other side of the centromere i.e. in the equal arm the first chiasma which is proximal to the centromere, the cross-like attachment of two chromatids of sister chromosomes (upper and lower one here) is clearly visible (Fig. 2).

Metaphase and anaphase I. 50 PMCs were analysed at metaphase I while counting the chiasma in this bivalent. Those in the equal arms have been ignored as they are not relevant to the present investigation. In these

50 heteromorphic bivalents only 4 have one chiasma in the unequal arm and the remaining 46 do not show any chiasma in the heteromorphic segment at metaphase I. It is significant that in most of the bivalents chiasma is not seen in the heteromorphic segment at metaphase I. It has already

Figs. 1-4a. 1, root tip squash preparation showing 32 chromosomes. Heteromorphic pair lying at 5 o'clock position. About 3000 $\times$. 2, heteromorphic bivalent at diplotene stage, showing 2 chiasmata in equal arms and one chiasma in unequal arms. Proximal chiasma in the equal arms showing a cross-like structure. About 3600 $\times$. 3, anaphase I, showing equational separation. About 3000 $\times$. 4, metaphase II, showing unequal chromosome, which is the consequence of equational separation at anaphase I of the heteromorphic bivalent. About 4000 $\times$. 4a, same enlarged. About 6000 $\times$.

been mentioned that at diplotene in every case where analysis was possible one chiasma was invariably seen in the unequal arm. At anaphase I clear analysis was made in 25 PMSs; in 16 of them the separation was equational

(Figs. 3, 4 and 4a) and in the remaining 9 reductional (Fig. 5). In several cases when all other chromosomes had reached the poles at early telophase the complements of the heteromorphic bivalent showing clearly equational separation was still at the equator (Figs. 6 and 6a).

Figs. 5-7b. 5, anaphase I, showing reductional separation. About 4000 $\times$. 6, late anaphase I, showing heteromorphic bivalent with equational separation, and as a laggard. About 1600 $\times$. 6a, same (heteromorphic bivalent enlarged). About 5000 $\times$. 7a, diplotene diagram of the heteromorphic bivalent if there had been literal crossing over of chromatids (which is not found). 7b, diplotene diagram of the heteromorphic bivalent supporting the partial chiasma type hypothesis (as seen in *Belamcanda chinensis*).

Discussion

The chromosome configuration at diplotene in this heteromorphic bivalent shows that the sister chromatids are together before and after the point of chiasma formation. If chiasma could have been the cause of crossing over the configuration should have been as shown in Fig. 7a i.e. the sister chromatids should have been together in the proximal region and non-sister chromatids in the distal region. In this particular case it would mean that in regions after the chiasma (on both sides) there should have been one longer and the other shorter chromatids i.e. non-sister chromatids should have been associated. This is not found. The only interpretation would then be that crossing over (break and union) has occurred between the non-sister chromatids before and the cross-like structure the chiasma, that is visible, is the

result of that event (Fig. 7b). There is thus no literal crossing over of chromatids i.e. no chiasma formation before genetical crossing over occurs.

Secondly, according to Mather's formula which has been found true in all the cases of equational and reductional separation upto $n=3$ (where n represents the number of chiasma) the percentage of equational separation of the loci after the point of chiasma would be 100% in case of $n=1$. Here, however, we find the equational separation is 64% and reductional 36%. There is an apparent discrepancy in the percentage of equational and reductional separation at anaphase I and frequency of chiasma at metaphase I. On the basis of chiasma frequency at metaphase I there would have been 92% reductional and only 8% equational separation. Because in every case where there is no chiasma in the unequal arm at metaphase I the separation would be reductional. This discrepancy can, however, be reconciled if the configuration at diplotene is taken into account where in every case we found a chiasma in the unequal arm. It therefore seems that between diplotene and metaphase I, in most cases, the chiasma slips off and what we see at metaphase I is not a true picture of what has happened at pachytene and diplotene. On this basis the anaphase data and diplotene configurations are more to be relied upon than the configurations at metaphase I. In this light it would be assumed that in the 64% of cases chiasma is always formed in the unequal arm and in the remaining it fails to occur. In every case of the occurrence of a chiasma, Mather's formula for $n=1$ would hold good.

Summary

In course of cytological investigation a heteromorphic pair of chromosomes was observed in one of the plants *Belamcanda chinensis* growing in the garden.

The pairing behaviour of the heteromorphic pair was studied in detail from the onset of meiosis.

At diplotene one chiasma was invariably observed in the heteromorphic arm of this bivalent but at metaphase I in many cases this bivalent did not show any chiasma in that arm, presumably the chiasma has slipped off.

The frequency of reductional and equational separation studied at anaphase I conformed to the Mather's formula for $n=1$.

The diplotene configuration of this bivalent supports the partial chiasma type theory of crossing over.

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