Evolution of Caryotype in an Interesting Species of Grasshopper, *Tristria pulvinata* Uvarov

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The diploid number in male Cryptosacci grasshoppers is typically 23 chromosomes. However, deviations from this are not uncommon. In *Aidemona azteca* (White 1951) the male individuals possess 21 chromosomes which could be accounted for by the presence of one pair of metacentric chromosomes in place of four acrocentric ones. Two or three centric fusions leading to the reduction in chromosome number have also been occurred in *Aleuas vitticoli*, *A. lineatus* (Saez 1932, 1935, 1945), and in different species of *Myrmeleotettix* and *Chorthippus* (see White 1954).

Centric fusions between the X-chromosome and the autosome leading to the reduction of spermatogonial chromosome number and the formation of neo-X and neo-Y mechanism have also been reported by various workers in a number of species of grasshoppers belonging to different genera (see Saez 1963, Manna and Chatterjee 1963).

Though centric fusion has been found to be the cause of reduction of chromosome number in many genera of grasshoppers referred to above, there are also reports where the chromosome number has been reduced without any apparent fusion of chromosome arms. In the genera *Miramella*, *Zubowskyia* and *Niitakacris*, the diploid chromosome number in males is 21 (Corey 1938, Helwig 1942, Momma 1943). An extreme loss of chromosome arms without centric fusion has been reported in *Dactylotum bicolor* (Helwig 1942, Powers 1942) where at least three pairs of regular autosomes have been eliminated from the male caryotype. On the other hand, a case of dissociation has recently been reported in *Moraba viatica* by White, Carson and Cheney (1964).

Dutt (1948) reported that in male *Tristria pulvinata* the diploid number of chromosomes is twenty one. By comparing the caryotype of some related genera he only mentioned the probable pair of autosomes missing in *T. pulvinata*. In the same species during the course of cytological investigation from a different population three types of individuals possessing three spermatogonial chromosome numbers such as 21, 22 and 23 chromosomes were observed (Manna and Mazumder 1965). As already mentioned the spermatogonial chromosome number in Cryptosacci grasshoppers is typically 23, and one or two chromosomes lacking in individuals of *T. pulvinata* with 22 and 21 chromosomes respectively are to be referred to hereunder as degenerated (D) chromosomes. However, on the contrary, if the basic number is considered to be 21 chromosomes, in that case individuals having 22 and 23 chromosomes could be called as individuals possessing one and two 'supernumerary' chromosomes respectively. In the previous communication (Manna and Mazumder 1965) this point was left open. In the present paper from the studies of spermatogenesis of individuals having 21 chromosomes and 23 chromosomes evidences would be forwarded as to the nature of the particular pair of chromosomes and the probable trend of evolution of the carotype in *T. pulvinata*.

Material and method

Adult male individuals constituting the material for the present study were collected in two successive years, 1964 and 1965 from the campus of the Kalyani University Agriculture College at Haringhata, West Bengal. Cytological constitution of each individual was determined mainly from permanent squash preparations. However, in order to extend the data quickly temporary aceto-carmine preparations were also used at a later period for the present study.

Observations

Meiosis in individuals with twenty-one chromosomes. The spermatogonial metaphase complement consisted of 21 acrocentric chromosomes in which the X-chromosome could not generally be distinguished from the other autosomes (Fig. 1).

The general course of meiosis was of orthodox type. At leptotene stage a large

Figs. 1-8. 1-5. Chromosomes of individuals with 21 chromosomes. Camera lucida drawings. $\times$ ca. 1300. 1, spermatogonial metaphase. 2, leptotene nucleus. 3 diplotene stage. 4, metaphase I. 5, anaphase I. 6-7. Chromosomes of individuals with 22 chromosomes. Camera lucida drawings $\times$ ca. 1300. 6, spermatogonial metaphase. 7, spermatogonial anaphase. 8, diplotene nucleus, D chromosome being free from the X-chromosome.

positively heteropycnotic mass represented the X-chromosome (Fig. 2) and at diplotene stage there were ten autosomal bivalent and the univalent heterochromatic X (Fig. 3). The X-chromosome at metaphase I remained more frequently at the equatorial region (Fig. 4) than forming an accessory spindle. First spermatocyte anaphase was reductional (Fig. 5) and the second anaphase was equational for the chromosomes.

Meiosis in individuals with twenty-two chromosomes. The spermatogonial metaphase complement consisted of 22 acrocentric chromosomes namely, 20 regular autosomes, one D (degenerated) chromosome and the X-chromosome. The staining behaviour of the D chromosome was erratic and in some spermatogonial metaphase complements it appeared to be a negatively heteropycnotic element (Fig. 6). Out of a limited number of spermatogonial anaphase cells available for the study no chromosome was seen to be lagging behind at this stage (Fig. 7). This fact suggested that the D chromosome was mitotically stable.

The D chromosome like the X-chromosome, however, appeared to be positively heteropycnotic at the first spermatocyte prophase stage, and its identification prior to diplotene stage was not very convincing since the nucleus sometimes contained some other heteropycnotic mass of this size. The D chromosome could be detected clearly from the diplotene stage onwards. At diplotene stage there were ten bivalents and the two deeply stained rod-like univalent chromosomes of unequal lengths (Fig. 8). The longer one was the X and the shorter one the D chromosome. The D might remain free from (Fig. 8) or connected with the X-chromosome by a thin chromatin thread-like connection (Fig. 9). At spermatocyte metaphase stage of first meiotic division the D was found to be precociously at one of the spindle poles (Figs. 10, 11). The segregation of the D and the X-chromosome at anaphase I was found to be random as already indicated by their positions in the metaphase I plates (Figs. 10, 11). First spermatocyte anaphase, like other chromosomes, was invariably reductional for the D chromosome. As a result of random segregation of the X and the D, four types of second spermatocyte metaphase cells were produced, e.g., 1) cells with 10 autosomes only (Fig. 12), 2) cells with 10 autosomes, the X and the D (Fig. 13), 3) cells with 10 autosomes and the X (Fig. 14) and 4) cells with 10 autosomes and the D (Fig. 15). The last two types of cells were similar as regards their chromosome numbers but the X and the D could be identified because of their size differences. Second spermatocyte anaphase was equational for all the chromosomes (Fig. 19). Hundreds of anaphase II cells showed regular disjunction of all the chromosomes. It indicated, therefore, that the centromere of the D chromosome was normal.

Meiosis in individuals with twenty-three chromosomes. The spermatogonial metaphase complement showed 23 acrocentric chromosomes comparable to the typical condition found in *Cryptosacci* grasshoppers. However, it consisted of 20 regular autosomes and the X like those of 21 chromosome bearing individual and in addition the two D chromosomes (Fig. 17). The identification of the D

chromosomes from their negatively heteropycnotic behaviour was not possible from the limited number of spermatogonial metaphase cells available for the study. Only a few spermatogonial anaphase cells could be observed. No abnormality in the segregation of the chromosomes was encountered.

At leptotene stage sometimes two extra positively heteropycnotic chromosomes

Figs. 9-16. Chromosomes of individuals with 22 chromosomes. Camera lucida drawings. $\times$ ca. 1300. 9, D chromosome attached to the X-chromosome at diplotene stage. 10, metaphase I stage, D and the X-chromosomes moving to one pole. 11, metaphase I, D chromosome and the X-chromosome polarised in the same pole. 12, metaphase II polar view with 10 autosomes only. 13, metaphase II, polar view with 10 autosomes plus the D and the X. 14, metaphase II, oblique view, 10, autosomes plus the X. 15, metaphase II, polar view, 10, autosomes plus the D. 16, anaphase II.

of equal size could be demarcated besides other such bodies (Fig. 18). These two heteropycnotic bodies most likely representing the two D chromosomes remained either separate (Fig. 18) or close to each other (Fig. 19). At the zygotene-

Figs. 17-24. Chromosomes of individuals with 23 chromosomes. Camera lucida drawings. $\times$ ca. 1300. 17, Spermatogonial metaphase with 23 chromosomes. 18, leptotene nucleus with three positively heteropycnotic bodies. 19, leptotene nucleus, the heteropycnotic bodies close to each other. 20, zygotene-pachytene nucleus. 21, diplotene nucleus, D bivalent with interstitial chiasma. 22, diplotene nucleus with twelve elements. 23, metaphase I side view 24, anaphase I.

pachytene stage the two D chromosomes could be identified from their close association showing the associated parts positively heteropycnotic while the unasociated parts were stained like the normal autosomes (Fig. 20). The nature of association of the two D chromosomes could be clearly followed at diplotene stage when they were found usually to be held by a chiasma. Further, this particular bivalent was positively heteropycnotic unlike other bivalents (Fig. 21). The position of the chiasma could either be interstitial (Fig. 21) or terminal (Fig. 22). In a quantitative study of 110 diplotene cells, the D chromosomes had interstitial chiasma in 45 cells and terminalised chiasma in 65 cells. In some diplotene nuclei, one of the bivalents other than the D-bivalent, showed almost half of it deeply stained (Fig. 21). However, the behaviour of the particular bivalent had not been followed for the present.

At diakinesis and metaphase I, the D-bivalent almost always had the chiasma in a terminalised condition. At metaphase I the D-bivalent could not be distinguished from other regular bivalents (Fig. 23). Anaphase I was always reductional for the Ds like the other members of the complement resulting in a distribution of 11 and 12 chromosomes in the two daughter cells (Fig. 24). A large number of anaphase I cells was studied but in no case were the two Ds found to be moving to the same pole. Further, out of a large number of anaphase II cells no irregularity in the segregation of the D chromosomes was observed when they divided invariably equationally. This regular disjunction of the Ds would indicate that they were regular members of the caryotype of *T. pulvinata* rather than supernumerary chromosomes.

Metrical studies of the chromosomes. In order to determine the size of the D chromosome, metrical studies of the second spermatocyte metaphase cells of individuals with 21 chromosomes and individuals with 22 chromosomes were made (Table 1). All the chromosomes have been classified according to their sizes into three main groups—long, medium and short. The X-chromosome stands in between the long and the medium size classes. The reason for taking metaphase II complements of individuals with 21 and 22 chromosomes was to obtain random samples and to see if there were any marked variation in the data taken from two sets of individuals.

It would appear from the data (Table 1*) that the D chromosome is the last member of the medium sized chromosomes i.e., its position is ninth in serial order of autosomes. However, the difference in sizes between the autosome eighth (even seventh) and the D chromosome was not at all appreciable and therefore, the D chromosome could very well be any one of them. To avoid complication the ninth chromosome has been designated hereafter as the D chromosome although it may be any one ranging from seventh to ninth in order of size.

In individuals with 22 chromosomes the D and the X-chromosome remained at metaphase I as univalents and rod-like in structure. So the measurement of 20 X-chromosomes and D chromosomes which was taken from the same metaphase I

stage and the mean values in micra were 6.3 and 3.0 respectively. The X/D ratio was found to be about 2:1. But the same ratio was found to be slightly altered at metaphase II stage if the mean value of the D chromosome was considered to be 1.9 micra (Table 1). This could be due to differential desprialisation of the chromosomes at this stage or else, the D chromosome could be the seventh or eighth autosome, as stated already, in order to size in which case the ratio of X/D was almost at par with that of metaphase I stage.

Table 1. Mean length in micra of the chromosomes of 40 metaphase II cells in each of the two types of individuals with 22 and 21 chromosomes

Size class	Serial No. of autosomes	Individuals with 22 chromosomes	Individuals with 21 chromosomes
Long	1	5.4	5.4
	2	4.7	4.7
	3	3.6	3.6
Medium	4	3.2	3.3
	5	2.8	3.0
	6	2.6	2.6
	7	2.3	2.0
	8	2.2	2.0
	9* (D chromosome)	1.9	missing
Short	10	1.4	1.3
	11	1.1	1.2
	X-chromosome	4.7	4.3

Table 2. Frequency distribution of individuals with different karyotypes in two successive years

Year	No. of indiv. with 21 chr.		No. of indiv. with 22 chr.	No. of indiv. with 23 chr.	Total	Gametes	
						T	t
1964	Obs.	163	17	11	191	0.90	0.10
	Exp.	154.71	34.38	1.9			
	Obs. %	85.35	8.90	5.75			
1965	Obs.	99	13	—	112	0.94	0.06
	Exp.	98.96	12.64	0.40			
	Obs. %	88.39	11.61	—			

Frequency of D chromosome in natural population. The frequencies of individuals having 21, 22, and 23 chromosomes had been determined in two successive years from the same population (Table 2).

An analysis of the data (Table 2) would reveal that in the sample collected in 1964 the number of individuals with 23 chromosomes was much higher than the expected number according to Hardy-Weinberg equilibrium. However, this wide discrepancy was not obtained in the sample collected in 1965. There might be several causes for the variance of the data which would be discussed later on.

Further, the high percentage of individuals with 21 chromosomes was quite agreeable in 1965 than in 1964 according to Hardy-Weinberg Law.

Discussion

The moot point on which the present investigation would throw light is on the question whether the particular chromosome referred to above as the D chromosome is either the supernumerary or a degenerating one. White (1954) is of opinion that regular chromosome of a caryotype may under special circumstances degenerate into a supernumerary chromosome. Thus the designation of the D chromosome as supernumerary would be a matter of personal likings of different workers since the Ds may turn out to be supernumerary by their behaviour. However, we have tried to separate Ds from supernumerary in some strict sense. It may be put in another way, if it were the case of degeneration, individuals with 22 and 21 chromosomes would represent monosomic to nullosomic condition for that particular chromosome. Monosomic and nullosomic individuals are common in plant species but relatively rare in animals.

Acrocentric nature and size of the D chromosome. The chromosomes of Acridids are characteristically acrocentric type. The supernumerary chromosomes whenever detected in the Family Acrididae are almost always acrocentric type (see White 1954, Manna 1954, Ray-Chaudhuri and Manna 1951, Nur 1963, Sannomiya 1962, John and Hewitt 1965). However, the size of the D chromosome in *T. pulvinata* is larger than at least two regular autosomes (Table 1), if not more, and its position varies between seventh and ninth chromosome in serial order. The supernumerary chromosomes are generally smaller in size (Battaglia 1964). Therefore, this fact would provide evidence for the consideration that the D chromosome was once a regular autosome in *T. pulvinata*.

Heterochromatic behaviour, chiasma formation and regularity in centrometric division. Almost complete heterochromatic nature of the D chromosome would indicate that this is a supernumerary chromosome since the B chromosomes in both animals and plants are in most cases heterochromatic in constitution (Battaglia 1964). However, heterochromatic transformation of some regular euchromatic chromosomes is not altogether rare (Muller 1932). Thus heterochromatic transformation of euchromatin in grasshoppers has been met with in a number of cases in the formation of the neo-X and neo-Y mechanism (see Saez 1963, Manna and Chatterjee 1963).

As reported already the D chromosomes form regular pairing, when two are present, during meiosis. The pairing of the two supernumeraries although very rare (Ray-Chaudhuri and Guha 1955) has been reported in *Trimerotropis sparsa* (White 1951). But unlike in *T. sparsa* where the two supernumeraries move frequently to one pole, the segregation of the particular pair of D chromosomes of *Tristria pulvinata* to opposite poles was as regular as any other pair of autosomes. This fact clearly indicates that unlike supernumerary chromosomes the centromere of the D chromosome is not defective at all. Irregularity in the behaviour of cent-

romeric division has been reported in the supernumerary chromosomes of *Aiolopus* sp. (Ray-Chaudhuri and Manna 1951, Ray-Chaudhuri and Guha 1955). Irregular segregation of the supernumerary chromosomes leads frequently to the intraindividual variation of chromosome numbers. In the present case no individual out of a total of 303 specimens examined cytologically has been observed with chromosome number beyond 23, i.e., not more than two Ds have been found to occur. This fact strongly supports the contention that the particular chromosome in *T. pulvinata* is possibly the degenerating chromosome and not the supernumerary one, or in other words the basic chromosome number in *T. pulvinata* has been 23 in males and, therefore, we are dealing with nullosomic and monosomic individuals for that particular chromosome in individuals with 21 and 22 chromosomes respectively. The partial euchromatic segment of the D chromosome in which probably the chiasma is formed indicated that there might be still some active genes present in that particular segment and the D chromosome has not been fully transformed genetically inactive.

Apparent absence of structural rearrangement. A comparison of the data of metrical study of the chromosomes of individuals of *T. pulvinata* with 21 and 22 chromosomes indicates that no gross structural change has taken place as a whole and in particular involving the X-chromosome among different chromosome forms. However, some occasional association between the D chromosome and the X chromosome in *T. pulvinata* like in some other species with supernumerary chromosomes (Ray-Chaudhuri and Manna 1951) might lead one to think of their homology, but this temporary association of the non-homologous chromosomes is anything but indicating a real homology and it is probably due to heterochromatin attraction as shown by Ribbands (1941) and by Ray-Chaudhuri and Manna (1950). Further, the D chromosome has never been seen to form multivalent structure in conjunction with other regular autosomes. This is further supported from the comparative data of Dutt (1948) collected on two related genera *Oxya* and *Spathosternum*. So it is observed that the origin of this particular chromosome is not evidenced at present due to any structural rearrangement. In this point apparently the present finding is not at par with White's (1954) view that no chromosome can simply degenerate without any structural rearrangement. However, there is no way to disprove altogether the absence of structural rearrangement involving the transfer of some vital genes to some other chromosome(?) in the long past, the indication of which no longer exists at present.

Probable origin. We have so far shown that evidences have not been obtained in support of considering the origin of Ds as due to some structural rearrangement. The origin, then, could be assumed in the following way. A mutation in this particular chromosome occurred and thereby changing it to heterochromatic transformation. The process of heterochromatinization by recessive and dominant mutations have been put forward by Muller (1932) and Fernandes (1946, 1949). In the present case the heterochromatinization would be the first step towards the

gradual loss of the ninth chromosome from the caryotype of *T. pulvinata*. In some later period there was some disturbance in the proper disjunction and thereby leading to twenty two and twenty one chromosome bearing individuals. In this transformation one point is to be considered. What would then be the condition of the genes contained in this particular chromosome for this transformation and gradual elimination? If this chromosome simply degenerates into inactive member, it might be envisaged that the genes contained in this particular chromosome were not very active and the elimination of which was not at all detrimental for the survival of the species. So, it could be said in another way that all the chromosomes of an individual are not essential for the survival for all the times. Such type of elimination of a whole chromosome has been observed in *Drosophila*. It may further be speculated for the present that the elimination of the particular pair of chromosome seems to be adaptively advantageous in the evolution of caryotype of *T. pulvinata*. The particular D chromosome could have some genes which seem to be useless in the present ecological conditions. But without any genetic knowledge of the grasshopper such an explanation could be taken as a mere speculation.

Frequency of different types of individuals in natural population. Dutt (1948) reported previously in *T. pulvinata* of only 21 chromosomes in males, but the present population in which the study has been conducted is a different one. From the study of frequencies of different types of individuals from one population in the present study it is seen that 21-chromosome bearing individuals are more frequent than the other two types. This might be due to either, i) the individuals with 21 chromosome might have a quicker rate of development, ii) accidentally the 21-chromosome bearing individuals were collected in large number, or, iii) that there is some differential selection effect on these individuals than the other two types. The deviation of Hardy-Weinberg equilibrium by showing excess number of individuals with 23 chromosomes in 1964 was not discernible in 1965. However, from the present data at our disposal it is more likely that 21-chromosome bearing individuals are enjoying a selective advantage over the other two types. Or, in other words the ninth chromosome of *T. pulvinata* is on the process of gradual elimination from the normal caryotype making it a case of caryotype evolution in action.

Judging the various facts discussed above the D chromosome represents the degenerating ninth (or, seventh or, eighth ?) chromosome and its monosomic condition is present in individuals with 22 chromosomes and nullosomic condition is exhibited by individuals with 21 chromosomes. Further, it is more likely in the evolutionary sense that this particular chromosome did not carry any active gene which could be essential for the survivality of the species. It seemed more likely that the elimination of this chromosome is rather advantageous for the species.

Summary

1. In a total of 303 males of *Tristria pulvinata*, examined from a population in

two successive years, three types of cytologically polymorphic individuals were encountered.

2. The meiotic behaviour of the 21-chromosome bearing individual was orthodox type which on comparative metrical study revealed that the ninth (or seventh or eighth?) pair of autosomes, termed as D chromosomes, is absent in individual which represents the nullosomic condition of the D chromosome.

3. The 22-chromosome bearing individuals include one D or the monosomic D and the behaviour of which is regular but peculiar.

4. The meiotic behaviour of the 23-chromosome bearing individual, which includes the two Ds, is regular like that of the normal *Cryptosacci* grasshoppers.

5. Discussions have been made as to the frequency, nature and the evolutionary significance of the D chromosome.

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