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Pachytene Analyses in *Atylosia scarabaeoides* and *Cajanus cajan* × *A. scarabaeoides* Hybrid[1]

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This is the third in a series of papers dealing with our investigations on interrelationship of *Cajanus cajan* and *Atylosia* species as revealed by hybridization and pachytene analysis. In the previous communication we (Reddy 1981 a, b) dealt with the pachytene analyses of two erect species of *Atylosia*, viz., *A. lineata* and *A. sericea* and their hybrids with *Cajanus*. *A. scarabaeoides* is a twiner and shows close similarities with *Cajanus* differing slightly in its growth habit and physiogeny. Studies on somatic karyology of *Cajanus* and *A. scarabaeoides* (Reddy and De, in press) indicated that 6 out of 11 chromosome pairs are common to them. The present paper deals with the pachytene karyomorphology of *A. scarabaeoides* and *Cajanus* × *A. scarabaeoides* hybrid.

Materials and methods

The materials used in this investigation are *A. scarabaeoides* and F_1 of *C. cajan* (variety T_{21}) × *A. scarabaeoides*. The cytological techniques employed here have been described in an earlier report (Reddy 1981 a, b).

Observations

Pachytene morphology of *Atylosia scarabaeoides*

The complement is constituted of 1 median, 9 submedian and 1 subterminal chromosomes. The individual bivalent length varies from 59.3 μ to 26.7 μ. The total chromatin length measures 431.8 μ of which 28.7 per cent is heterochromatic. Chromosomes I and II belong to the long group, chromosomes III to IX to the medium group and chromosome X and XI to the short group.

The data on the mean total length, arm length and arm ratio of the individual bivalents are summarised in Table 1 and the idiogram is given in Fig. 10.

The characteristic features of each bivalent are detailed below:

Chromosome I (Figs. 1A and 1B) is the longest of the complement. Nearly one third of the long arm and one-fifth of the short arm are made of HC (long arm 10.6 μ; short arm 5.7 μ).

Chromosome II (Figs. 2A and 2B) is submedian. The HC (10.2 μ) of the long arm

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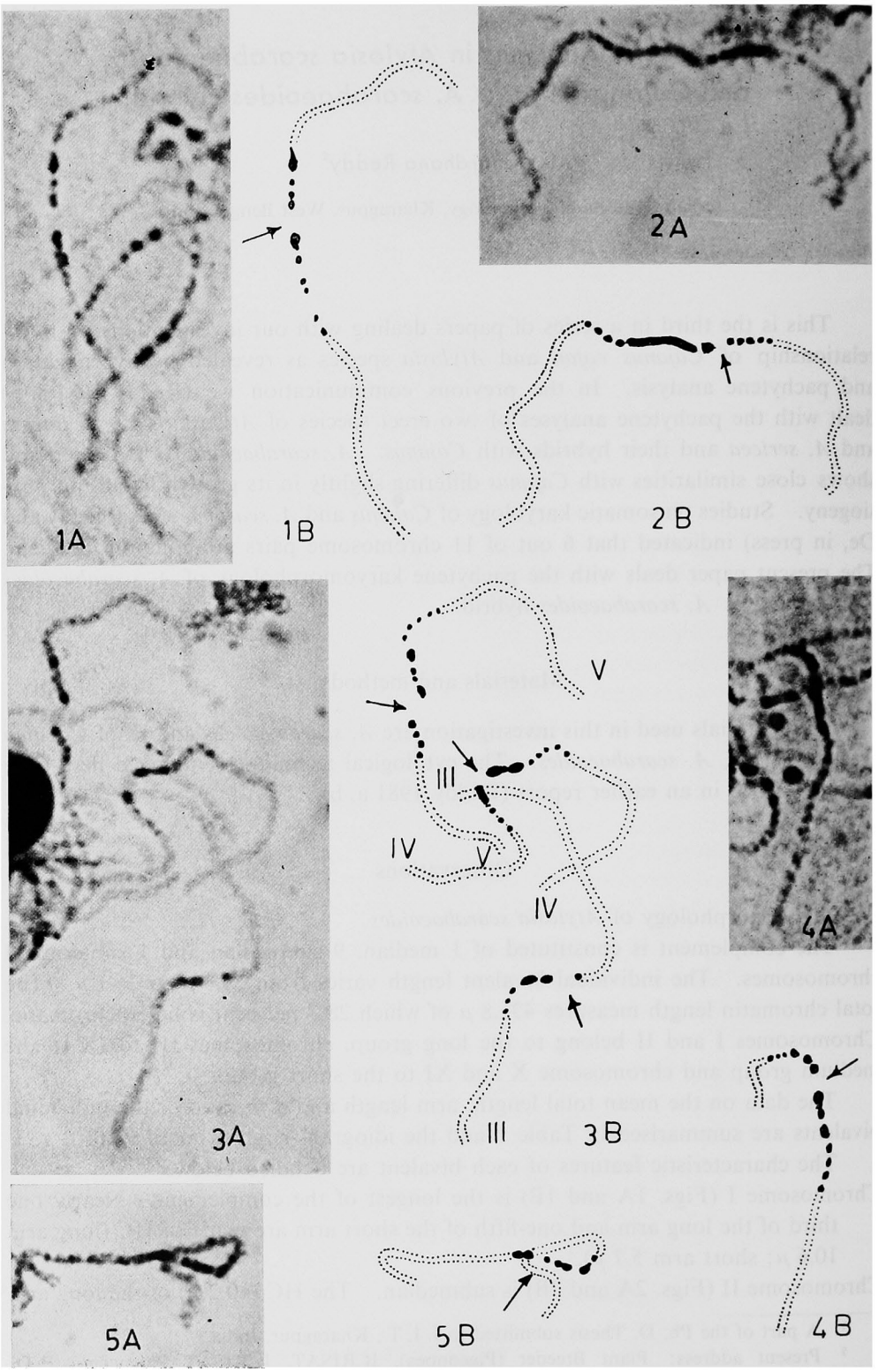
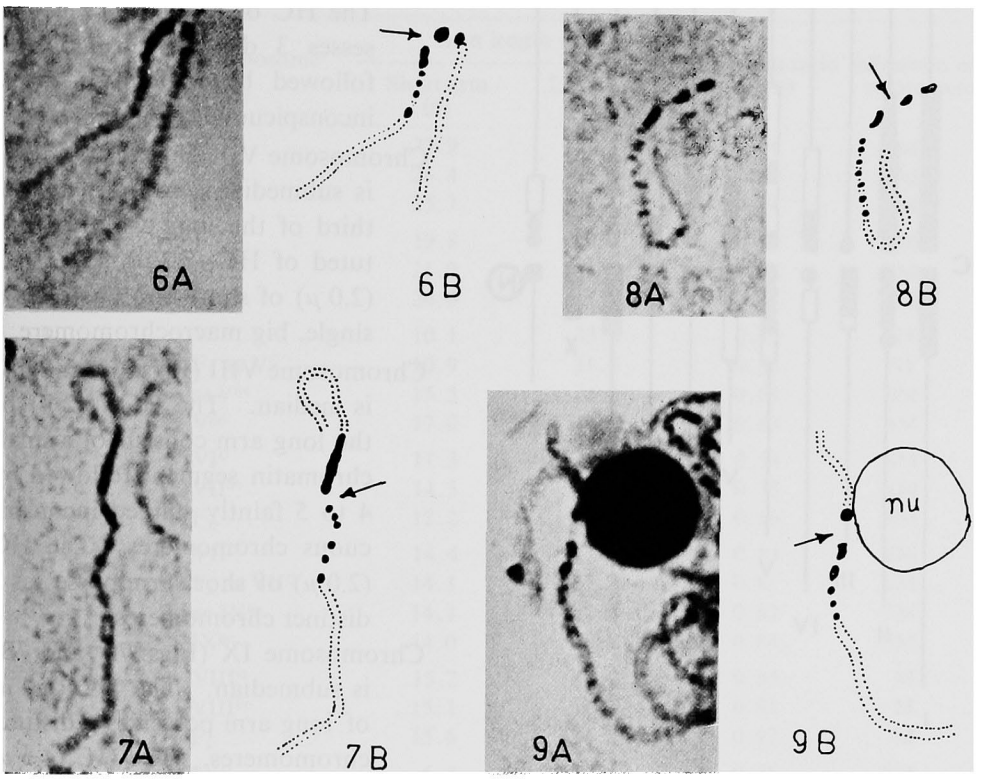


Figs. 1–5. *Atylosia scarabaeoides* pachytene chromosomes. In each pair of figures A is a photomicrograph (×2000) and B is an interpretive drawing. Arrows indicate position of centromere. 1A and B, chromosome I. 2A and B, chromosome II. 3A and B, chromosomes III, IV and V. 4A and B, chromosome VI. 5A and B, chromosome VII.

is twice to that of the short arm and consists of 5 distinct deeply stained chromomeres of which proximal one is separated from the rest by a less deeply stained chromatin segment. One-fourth of the short arm is made of HC (5.1 μ) and possesses 5 equal-sized distinct chromomeres.

Chromosome III (Figs. 3A and 3B) is submedian. The HC (1.0 μ) of long arm consists of a single distinct chromomere. The HC (4.0 μ) of short arm possesses 2 closely appressed macrochromomeres followed by 2 to 3 small, inconspicuous faintly stained chromomeres.



Figs. 6–9. *Atylosia scarabaeoides* pachytene chromosomes. In each pair of figures A is a photomicrograph (×2000) and B is an interpretive drawing. Arrows indicate position of centromere. 6A and B, chromosome VIII. 7A and B, chromosome IX. 8A and B, chromosome X. 9A and B, chromosome XI (nu=nucleolus).

Chromosome IV (Figs. 3A and 3B) is submedian. One-fourth of the long arm is made of HC (6.7 μ) which is twice as long as that (3.4 μ) of the short arm. The long arm consists of a chromatic segment followed by 5 to 6 faintly stained inconspicuous chromomeres. The short arm possesses a single macrochromomere followed by 3 faintly stained chromomeres.

Chromosome V (Figs. 3A and 3B) is submedian. The HC (10.3 μ) of long arm possesses 2 distinct chromomeres separated by 2 smaller faintly stained chromomeres. One-third of the short arm is made of HC (5.8 μ) and consists of 4 distinct chromomeres followed by faintly stained, inconspicuous chromomeres.

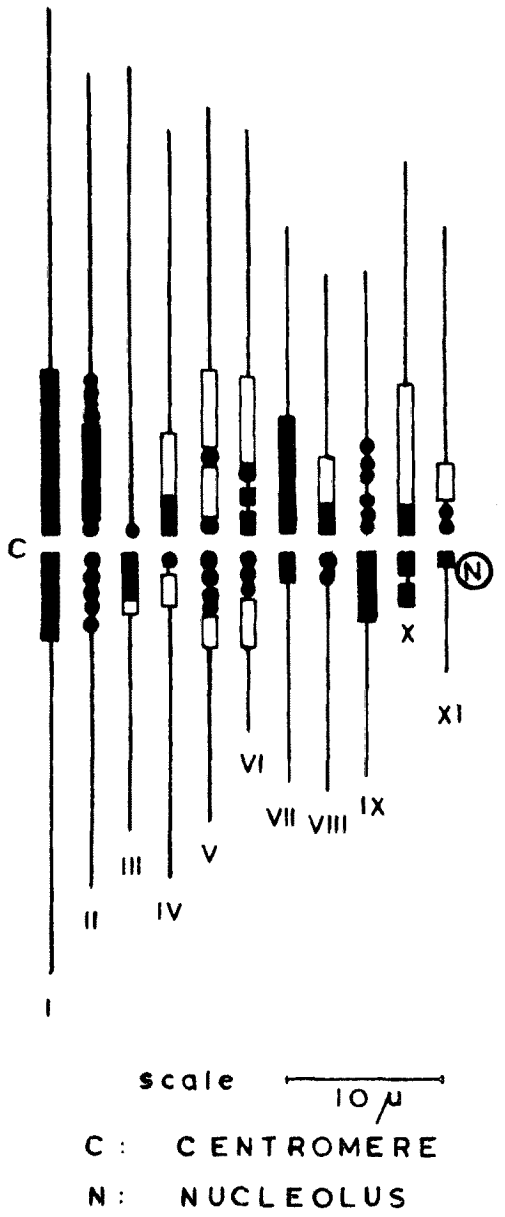


Fig. 10. Idiogram of the pachytene karyotype of *A. scarabaeoides*.

Chromosome VI (Figs. 4A and 4B) is submedian. Two-fifths of the long arm is made of HC (10.1 μ) and consists of 3 big barrel shaped chromomeres followed by 3 to 4 small chromomeres. The short arm is constituted of nearly equal lengths of HC (6.0 μ) and EC. The HC of the short arm possesses 3 distinct chromomeres followed by 3 faintly stained, inconspicuous chromomeres.

Chromosome VII (Figs. 5A and 5B) is submedian. More than one-third of the long arm is constituted of HC (7.3 μ). The HC (2.0 μ) of short arm possesses a single, big macrochromomere.

Chromosome VIII (Figs. 6A and 6B) is median. The HC (5.2 μ) of the long arm consists of a small chromatin segment followed by 4 to 5 faintly stained inconspicuous chromomeres. The HC (2.0 μ) of short arm possesses 2 distinct chromomeres.

Chromosome IX (Figs. 7A and 7B) is submedian. The HC (6.0 μ) of long arm possesses 6 distinct chromomeres. The HC (4.6 μ) of short arm consits of a darkly stained chromatic segment.

Chromosome X (Figs. 8A and 8B) is subterminal. The HC (9.3 μ) of the long arm consists of a small chromatic segment followed by a few very faintly stained chromomeres. The short arm is completely heterochromatic (3.4 μ) and consists of 2 barrel shaped chromomeres.

Chromosome XI (Figs. 9A and 9B) is a nucleolus organising chromosome. The nucleolus is located near the short arm and the satellite measures 4.6 μ. The HC (4.6 μ) of the long arm consists of 2 distinct macrochromomeres followed by 3 to 4 faintly stained chromomeres. The HC (1.0 μ) of short arm possesses a single distinct chromomere.

Pachytene morphology of *Cajanus* × *A. scarabaeoides* hybrid

Different degrees of synapsis was noticed in the hybrid during pachytene. In eight bivalents the homoeologues participating in pairing were identified. Out of these eight bivalents, one showed non-homologous association. In the remaining three bivalents, only one partner could be identified. A comparative statement of the cytological values of the parents and the hybrids is given in Table 1.

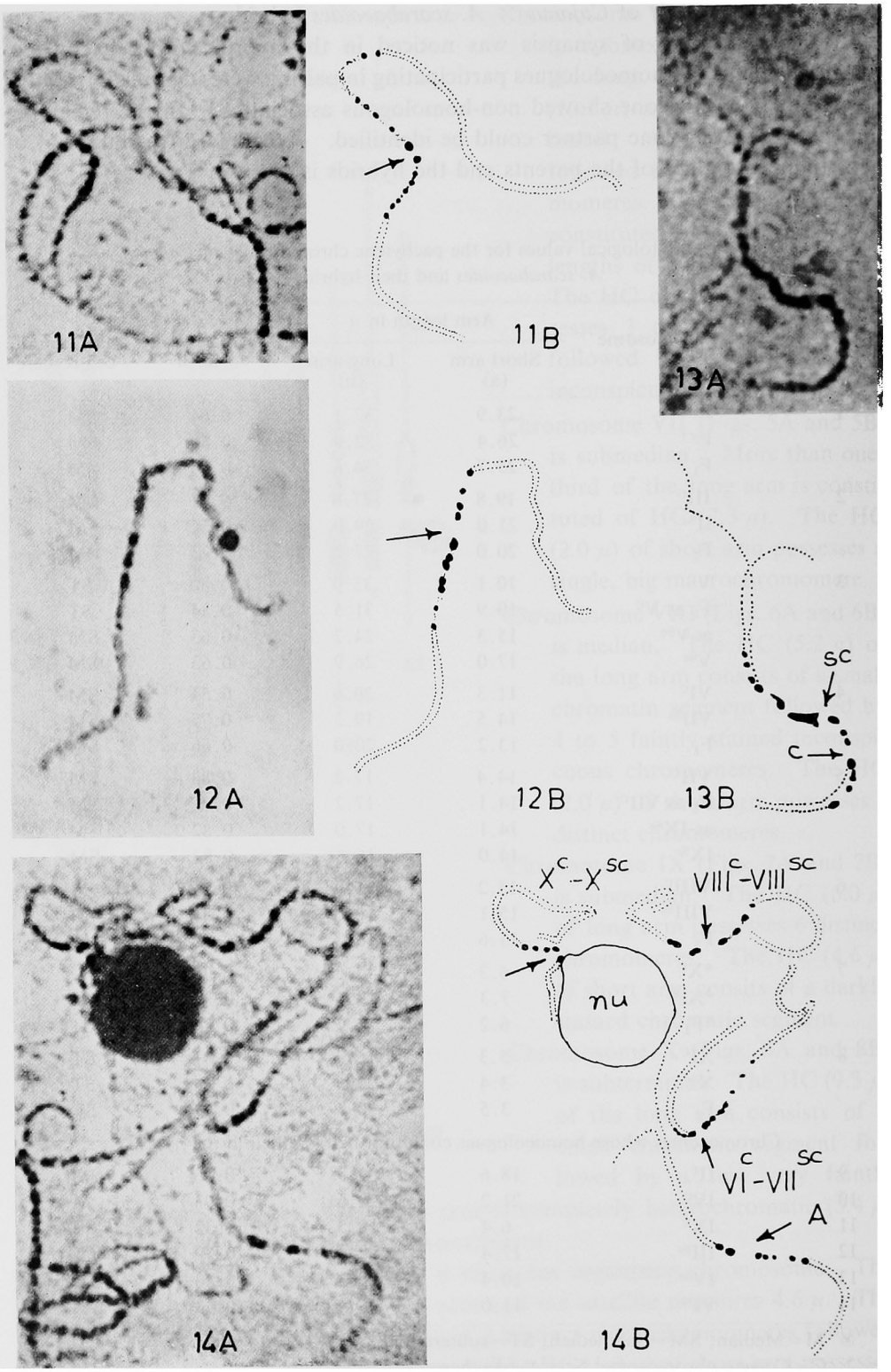
Table 1. Cytological values for the pachytene chromosomes of *Cajanus*, *A. scarabaeoides* and their hybrid

Sl. no.	Chromosome	Arm length in μ		Mean arm ratio (a/b)	⊙Position of centromere
		Short arm (a)	Long arm (b)		
1	I^{C+}	23.9	37.1	0.64	SM
	I^{Sc+}	26.4	32.9	0.80	SM
	F_1	25.7	34.6	0.74	SM
2	III^{C}	19.8	27.8	0.71	SM
	II^{Sc}	21.0	29.0	0.72	SM
	F_1	20.0	27.2	0.73	SM
3	V^{C}	10.1	33.0	0.30	ST
	F_1 as V^{C}	10.9	31.5	0.34	ST
	as V^{Sc}	15.3	24.2	0.63	SM
	V^{Sc}	17.0	26.9	0.63	SM
4	VI^{C}	11.3	20.6	0.54	SM
	VII^{Sc}	14.5	19.3	0.75	SM
	F_1	13.2	20.0	0.66	SM
5	VII^{C}	14.4	17.2	0.83	SM
	F_1 as VII^{C}	14.1	17.2	0.81	SM
	as IX^{Sc}	14.1	17.0	0.82	SM
	IX^{Sc}	14.0	16.6	0.84	SM
6	$VIII^{C}$	15.2	15.9	0.95	M
	$VIII^{Sc}$	15.1	16.6	0.91	M
	F_1	15.6	16.8	0.92	M
7	$*X^{C}$	6.3	17.5	0.36	SM
	$*XI^{Sc}$	7.3	19.4	0.37	SM
	$*F_1$	6.2	17.7	0.35	ST
8	XI^{C}	5.3	16.9	0.31	ST
	X^{Sc}	3.4	23.5	0.14	ST
	F_1	3.5	20.7	0.17	ST
	Chromosomes whose homoeologues could not be identified in the hybrid				
9	II^{C}	18.6	32.9	0.56	SM
10	IV^{C}	21.2	22.6	0.93	M
11	IX^{C}	6.4	19.7	0.32	ST
12	III^{Sc}	17.4	29.4	0.59	SM
13	IV^{Sc}	20.4	25.3	0.80	SM
14	VI^{Sc}	11.0	25.4	0.43	SM

⊙ M=Median; SM=Submedian; ST=subterminal,

\+ C=*Cajanus* chromosome; Sc=*A. scarabaeoides* chromosome

* denotes nucleolar chromosome.


11A
11B
13A
12A
12B
13B
sc
c
14A
14B
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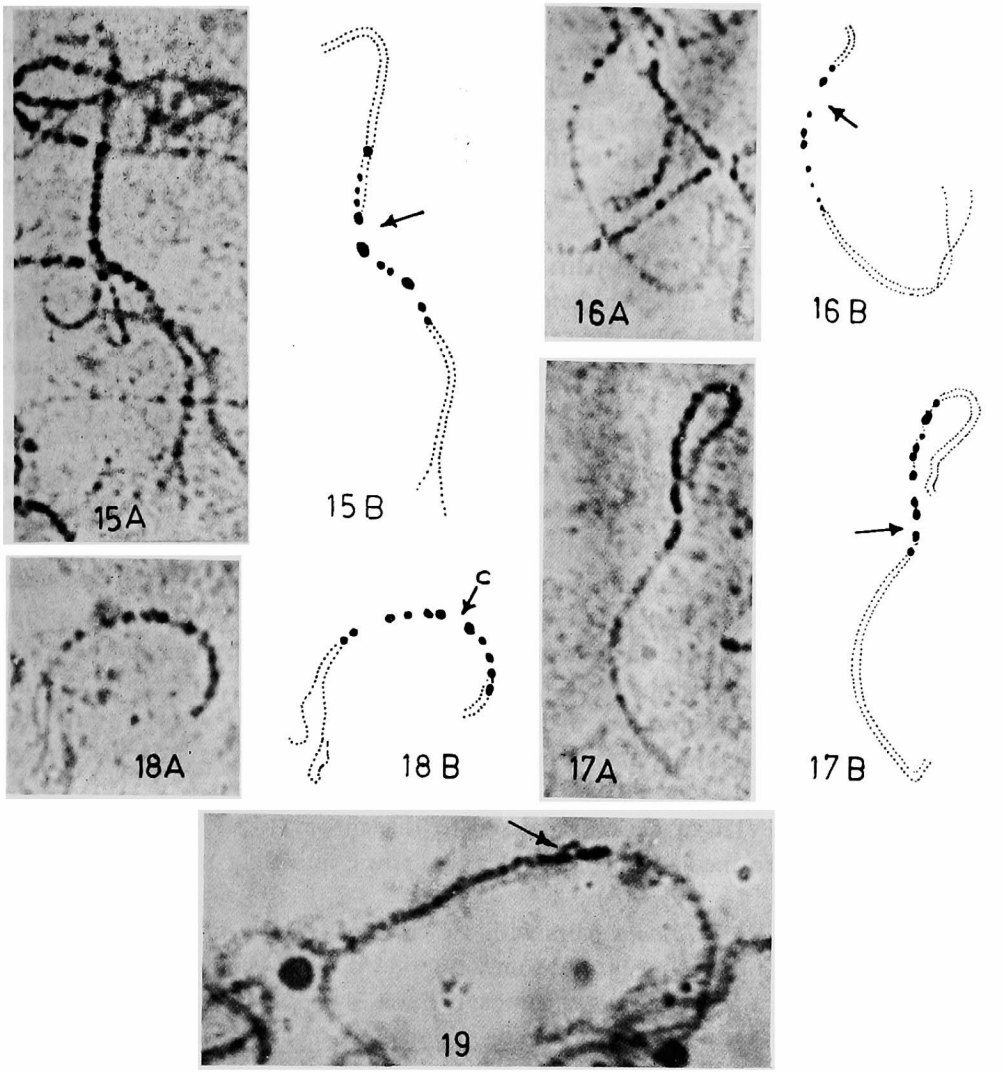
A



Figs. 15–19. Pachytene chromosomes of *C. cajan* × *A. scarabaeoides* hybrid. In each pair of figures A is a photomicrograph (×2000) and B is an interpretive drawing. Arrows indicate position of centromere. 15 A and B, bivalent VII^{C}–IX^{Sc}. Note heteromorphism in the long arm. 16 A and B, bivalent XI^{C}–X^{Sc}. Note failure of pairing in the EC of the long arm. 17 A and B, bivalent 'B'. 18 A and B, bivalent 'C'. See heteromorphism in the long arm. 19, A duplication buckle (Arrow).

Figs. 11–14. Pachytene chromosomes of *C. cajan* × *A. scarabaeoides* hybrid. In each pair of figures A is photomicrograph (×2000) and B is an interpretive drawing. Arrows indicate position of centromere. 11 A and B, bivalent I^{C}–I^{Sc}. 12 A and B, bivalent III^{C}–II^{Sc}. 13 A and B, bivalent V^{C}–V^{Sc}. Note non-homologous association of large parts including centromeres (C and Sc are centromeres of *C. cajan* and *A. scarabaeoides* respectively). Also note failure of pairing and heteromorphism in the long arm. 14 A and B, bivalents IV^{C}–VII^{Sc}, $VIII^{C}$–$VIII^{Sc}$, X^{C}–XI^{Sc} and bivalent 'A'. Note the heteromorphic nature of bivalent 'A' in its long arm and the nucleolus (nu) attached to the short arm of X^{C}–XI^{Sc} bivalent.

Chromosome I of *Cajanus* pairs with the chromosome I of *A. scarabaeoides* which has higher arm ratio and shorter long arm. This bivalent is completely paired inspite of the arm length differences of the parent chromosomes (Figs. 11A and 11B).

Chromosome III of *Cajanus* pairs with the chromosome II of *A. scarabaeoides* which has longer HC. In most of the microsporocytes, this bivalent is completely paired (Figs. 12A and 12B). In a few cells non-pairing in the HC region of long arm was observed.

Chromosome V of *Cajanus* pairs with the chromosome V of *A. scarabaeoides.* These two chromosomes differ in their length and arm ratio. Large parts including the centromere of the bivalent show non-homologous pairing (Fig. 13A and 13B). In most of the cells, the EC of long arm remains unpaired and exhibits heteromorphism.

Chromosome VI of *Cajanus* pairs with the chromosome VII of *A. scarabaeoides.* These two chromosomes vary in their arm ratio with *Cajanus* chromosome possessing a shorter short arm than that of *scarabaeoides* chromosome. The bivalent is intermediate to those of the parents in its arm ratio (Fig. 14A and 14B).

Chromosome VII of *Cajanus* pairs with the chromosome IX of *A. scarabaeoides.* The two chromosomes differ in the HC amount of short arm, *Cajanus* possessing a single macrochromomere in contrast to a longer chromatic segment of *scarabaeoides* chromosome. In the hybrid, this bivalent is readily recognised by the presence of 6 distinct chromomeres in its long arm. In most of the meiocytes, the distal EC region of the long arm is not paired and exhibits heteromorphism (Figs. 15A and 15B).

Chromosome VIII of *Cajanus* pairs with the chromosome VIII of *A. scarabaeoides.* Both are median chromosomes and are completely paired throughout their length (Figs. 14A and 14B).

Chromosome X of *Cajanus* pairs with the chromosome XI of *A. scarabaeoides.* Both are nucleolus-organising chromosomes but they differ in the number of chromomeres in the short arm. *Cajanus* chromosome possesses 2 distinct chromomeres in its short arm in contrast to a single chromomere in *scarabaeoides* chromosome. In the hybrid, normally this bivalent is paired throughout its length (Figs. 14A and 14B).

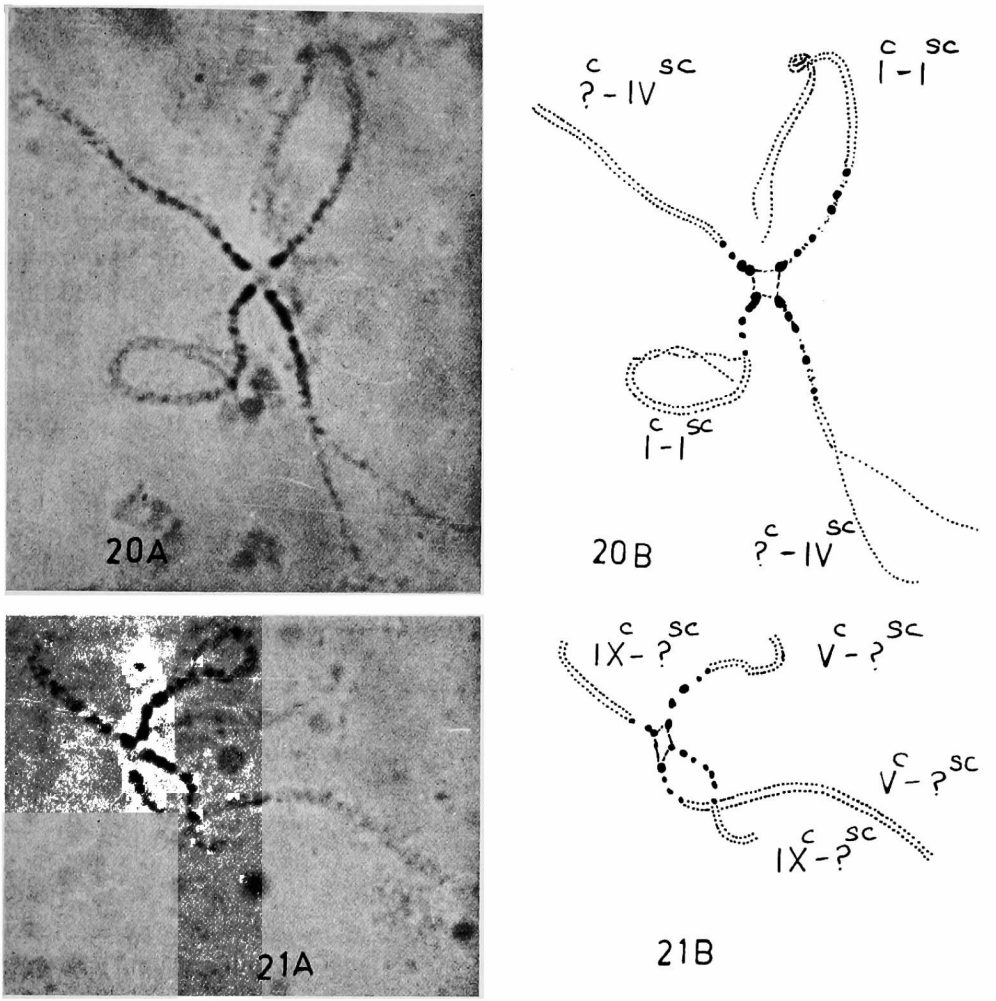
Chromosome XI of *Cajanus* pairs with the chromosome X of *A. scarabaeoides.* Both are subterminal chromosomes and *Cajanus* chromosome is slightly shorter with higher arm ratio. In the hybrid, the bivalent possesses an arm ratio nearer to *scarabaeoides* chromosome (Figs. 16A and 16B)

The bivalents formed by the chromosomes II, IV and IX of *Cajanus* and chromosomes III, IV and VI of *A. scarabaeoides* could not be identified. Instead, three bivalents which are morphologically distinguishable from each other were observed and only one partner participating in synapsis could be identified in each case. These bivalents are tentatively referred to as 'A', 'B', and 'C' and their morphology is detailed below:

Bivalent 'A' (Figs. 14A and 14B) was observed in 4 cells and is heteromorphic in its long arm. The short arm measures 12.0 μ and is completely paired. The long arms of this bivalent differ in length and measure 15.3 μ in one homoeologue and

26.2 μ in the other with an arm ratio of 0.78 and 0.41 respectively. The latter homoeologue with a total length of 38.2 μ and arm ratio of 0.41 resembles chromosome VI of *scarabaeoides*. But they do not agree in their HC pattern.

Bivalent 'B' (Figs. 17A and 17B) was seen in 3 cells. It measures 39.8 μ with



Figs. 20–21. Translocation configurations in the pachytene stage of *C. cajan* × *A. scarabaeoides* hybrid. In each pair of figures A is a photomicrograph (×2000) and B is an interpretive drawing. Arrows indicate position of centromere. 20 A and B, translocation-1 involving bivalent I^{c}–I^{sc} and chromosome IV of *A. scarabaeoides*. The *Cajanus* homoeologue in the second bivalent could not be identified (?). 21 A and B, translocation-2 involving chromosome V and IX of *Cajanus*. Their respective *A. scarabaeoides* homoeologues could not be identified (?).

an arm ratio of 0.93 and was found to be completely paired. It comes closer to chromosome IV of *Cajanus* in its length and arm ratio but differs in its HC pattern.

Bivalent 'C' (Figs. 18A and 18B) is heteromorphic and was observed in 4 cells. One of the homoeologue of this bivalent is identified as chromosome IX of *Cajanus* which measures 26.2 μ with an arm ratio of 0.33. It agrees with chromosome IX

of *Cajaus* even in its HC pattern. The *scarabaeoides* homoeologue could not be identified.

In a few microsporocytes two translocations and an interstitial duplication in one bivalent were noticed (Fig. 19).

In one translocation chromosome V and IV of *Cajanus* (Figs. 20 A and 20B) and in the other chromosome I and IV of *A. scarabaeoides* (Figs. 21 A and 21B) were involved.

Discussion

The pachytene chromosomes of *A. scarabaeoides* like the other two erect species of *Atylosia*, *A. lineata* and *A. sericea* (Reddy 1981 a, b) belong to the differentiated category. *A. scarabaeoides* possesses one median, nine submedian and one subterminal chromosomes as against two median, six submedian and three subterminal chromosomes in *Cajanus*. Seven chromosomes viz. I, II, III, VII, VIII, X and XI of *Cajanus* correspond with I, III, II, IX, VIII, XI, and X of *A. scarabaeoides* respectively. These corresponding pairs show varying degrees of similarity. While some are exactly identical in all the morphological criteria employed in their identification, some are identical only in few aspects.

As in *C. cajan* × *A. sericea* hybrid, two translocations and a duplication loop were observed in *C. cajan* × *A. scarabaeoides* hybrid also. The fact that in both these hybrids and not in *C. cajan* × *A. lineata* (Reddy, loc. cit.) chromosome V of *C. cajan* is involved in one of the translocations indicate that *A. sericea* and *A. scarabaeoides* are closer to each other and *A. lineata* is closer to *C. cajan*. This is further evidenced by the observation that while no duplications were noticed in *C. cajan* × *A. lineata* hybrid, in both *C. cajan* × *A. sericea* and *C. cajan* × *A. scarabaeoides* hybrids duplication buckles were commonly observed.

At pachytene stage, two bivalents, IV^{C}–VII^{L} and XI^{C}–XI^{L} showed heteromorphism in *C. cajan* × *A. lineata* hybrid. Similarly two bivalents i.e., IV^{C}–IV^{S} and IX^{C}–XI^{S} exhibited heteromorphism in *C. cajan* × *A. sericea* hybrid while in *C. cajan* × *A. scarabaeoides* 4 bivalents V^{C}–V^{Sc}, VII^{C}–IX^{Sc}, bivalent 'A' (unidentified) and bivalent 'C' (Chromosome IX of *Cajanus*; its *A. scarabaeoides* partner could not be identified) showed heteromorphism. The fact that different chromosomes of *Cajanus* exhibit heteromorphism in the different hybrids of *Atylosia* suggests that these species have followed separate evolutionary pathways for a considerable period. Similar observations where different chromosomes exhibited heteromorphism in two hybrids of *Lycopersicon esculentum* × *Solanum pennelli* and *L. esculentum* × *S. lycopersicoides* have been reported by Khush and Rick (1963). However, in the present study, the observation that the chromosome IX of *Cajanus* exhibits heteromorphism both in *C. cajan* × *A. sericea* and *C. cajan* × *A. scarabaeoides* hybrids points out that *A. sericea* and *A. scarabaeoides* are closer to each other than either of them to *A. lineata*.

In *C. cajan* × *A. scarabaeoides* hybrid, chromosome V of *Cajanus* pairs with chromosome V of *A. scarabaeoides* non-homologously for a larger part including centromeres. However in a few cells where chromosome V and IX of *Cajanus* are

involved in a traslocation, the *A. scarabaeoides* homoeologue of the former bivalent, although remains unidentified, is definitely not chromosome V of *A. scarabaeoides*. Thus it appears that in the hybrid, chromosome V of *Cajanus* enjoys a choice of pairing with either chromosome V or an unidentified one of *A. scarabaeoides*. Similar observation where single chromosome shows pairing associations with three different chromosomes was made by Chu (1967) in pachytene of haploid rice. Thus when an exact homologue or homoeologue is not present a chromosome may show non-homologous pairing with a chromosome which also lacks an exact partner. When more than 2 such chromosomes without their exact partners are present, the synapsis (if occurs) becomes a random process.

Summary

All the eleven pachytene chromosomes of *A. scarabaeoides* were identified on the basis of their relative length, arm ratio, chromomere pattern and nucleolar association. The pachytene complement of *A. scarabaeoides* consists of one median, 9 submedian and one subterminal chromosomes. On the basis of direct comparison of parental pachytene chromosomes, 7 chromosomes are common to *A. scarabaeoides* and *C. cajan*. In 8 bivalents of the hybrid the homoeologues participating in pairing were identified. Out of these 8, one bivalent showed non-homologous association and 4 were found to be heteromorphic. Two translocations and an interstitial duplication were noticed in a few microsporocytes. On the basis of the common chromosomes involved in translocations and those exhibiting heteromorphism in the 2 hybrids, *C. cajan* × *A. sericea* and *C. cajan* × *A. scarabaeoides* but not in the *C. cajan* × *A. lineata* hybrid it is concluded that *A. sericea* and *A. scarabaeoides* are closer to each other than either of them to *A. lineata*.

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