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Interspecific hybrids of *Antheraea roylei* and *A. pernyi* – a cytogenetic reassessment

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Summary. A rare case of interspecific hybridization between the Indian oak feeding silkworm *Antheraea roylei* (n=31) and Chinese oak feeding silkworm *A. pernyi* (n=49) yielding fertile and vigorous offspring is reported. The F_1 and the backcross (*A. roylei* × *A. pernyi*) × *A. pernyi* male individuals of the above cross and the F_{23} and F_{32} male offspring derived from an earlier cross between another race of *A. roylei* (n=30) and *A. pernyi* (n=49) were cytogenetically analysed in order to *study* their chromosome dynamics. The F_1 hybrids showed 18 trivalents and 13 bivalents in the first meiotic prophase and metaphase. The backcross individuals possessed either 9 trivalents and 31 bivalents or 49 bivalents, in Metaphase I cells. The F_{23} and F_{32} individuals were karyotypically alike and exhibited 49 bivalents. The following conclusions were drawn from the above observations: (a) in spite of allopatry and karyotypic divergence in number, a high degree of homology exists between the chromosomal complements of the two species; (b) *A. pernyi* possibly evolved from *A. roylei,* during the course of which 18 chromosomes of the latter underwent fission to give rise to the 36 chromosomes of the former. This is demonstrated by trivalent formation and pairing affinities in F_1 hybrids; (c) selection has favoured the elimination of large *A. roylei* chromosomes which participated in trivalent formation in successive generations of inbred hybrids to establish a stable Karyotype like that of *A. pernyi.*

Key words: Antheraea – Interspecific hybrid – Trivalents

Introduction

Since species are genetically closed systems with isolating mechanisms operating at different levels in various degrees, interspecific hybrids are rarely encountered in nature.

However, reports of *enforced* interspecific crosses leading to the production of hybrids are many (see White 1973) and although many of these hybrids are quasi or completely sterile, a fraction of them have been found to be fertile (Foot and Storbell 1914; Darlington 1939; Carothers 1941; Veshima 1963). Jolly et al. (1969) obtained fertile hybrids in a cross between a chromosomal race of *Antheraea roylei* (n=30) and *A. pernyi* (n=49). The cytogenetic analysis of the F_2 and backcross individuals showed "Chromosome configurations" of n=30 at F_1, 32, 42, 44 and 48 at F_2 and 34, 42, 46 and 49 in backcrosses with *A. pernyi*' (Jolly et al. 1979).

In light of these findings, the present investigation is concerned with: (a) the analysis of the first meiotic division with special emphasis on pairing properties and mode of segregation in hybrids obtained by the cross of another chromosomal race of *A. roylei* (n=31) with *A. pernyi* (n=49). This will have relevance on previous work (Jolly et al. 1979) where the *A. roylei* parent was observed to possess a haploid number of 30 chromosomes; (b) the meiotic study of backcross offspring of the above hybrids; (c) the chromosomal analysis of the male germline cells of F_{23} and F_{32} individuals derived from previous interspecific crosses (Jolly et al. 1979).

Materials and methods

The F_1 hybrids used in the present analysis were derived by crossing the n=31 race of Indian oak feeding silkworm *A. roylei* collected from Batote, Jammu and Kashmir State, India, with *A. pernyi* (n=49), which is of Chinese origin and

maintained at the State Government silk farm, Ramsu, Jammu and Kashmir State, India. The backcross offspring were obtained by crossing F_1 females with males of *A. pernyi*. The other sources of materials were the males of F_{23} and F_{32} offspring whose lineage can be traced to the hybrids of the n=30 race of *A. roylei* and *A. pernyi* (n=49) (Jolly et al. 1969) which are maintained at the commercial level at the Regional Tasar Research Station, Imphal, Manipur, India.

The testes of 5th instar larvae and early pupal stages were used for cytological observations. Slides were prepared by adopting the technique of Imai (1974). They were stained in Giemsa and then diluted 25 times in Sorenson's phosphate buffer (pH 6.8) for 25–30 min.

Results

Parents

Analysis of metaphase I plates of *A. roylei* revealed 31 bivalents to give diploid number of 62 (Fig. 1). Many of these bivalents were fairly large when compared to those of *A. pernyi*. Metaphase I plates of *A. pernyi* exhibited 49 bivalents reflecting a diploid number of 98 chromosomes, most of which were relatively smaller than those observed in *A. roylei* (Fig. 2).

A. roylei (n=31)×A. pernyi (n=49) hybrids

Both male and female F_1 hybrids were fertile and vigorous, and the cocoons were larger and harder than those of *A. pernyi*. The shape of the cocoons resembled those of *A. pernyi* in that they lacked the double layered nature of *A. roylei*. In general, the hybrids behaved in a heterotic manner. Male meiotic analysis revealed 31 elements in the Diplotene and Metaphase I stages (Figs. 3 and 4): in the latter 18 elements could be recognised as trivalents and the remaining 13 as bivalents (Table 1). Each trivalent exhibited complete pairing between one large *A. roylei* chromosome (R) and two small *A. pernyi* chromosomes (P). Thus, the two *Pernyi* chromosomes flanked the *roylei* chromosome on either side, being held in position by two chiasmata (Fig. 5).

Backcross offspring

The offspring obtained in the backcross of hybrid females with *A. pernyi* males showed only 40 or 49 chromosome elements in Metaphase I spermatocytes. When 40 chromosome elements were encountered, 9 trivalents were observed, each of which was similar to those found in F_1 individuals, and 31 bivalents (Fig. 6). When metaphase I plates contained 49 chromosomal elements, all were in the nature of bivalents. No other variation of chromosome elements was observed (Table 1).

Inbred F_{23} and F_{32} males of A. roylei (n=30)×A. pernyi (n=49)

The meiotic pattern and number of chromosomal elements were similar in F_{23} and F_{32} males. They

Table 1. Frequency of chromosome number at F1 (*A. roylei*×*A. pernyi*) (n=31×n=49) Backcross, F1 (*A. roylei* (N=31) – *A. pernyi* (n=49)), and F23 (*A. roylei* (n=30)×*A. pernyi* (n=49))

	No. of hybrid males cytologically scored	No. of cells examined	Chromosome no.	No. of trivalents	No. of bivalents
F1 *A. roylei*×*A. pernyi*	4	120	31	18	13
F1 (*A. roylei*×*A. pernyi*)×*A. pernyi*	2	40	40	9	31
	3	45	49	–	49
F23 (*A. roylei* (n=30)×*A. pernyi* (n=49))	42	406	49	–	49

Table 2. Possible segregation of chromosomes during spermatogenesis of a backcross between F1 ♀ and *A. pernyi* ♂

Gametic karyotypes		Diploid no. of backcross individuals	Metaphase I configurations	
F1	*A. pernyi*		Expected	Observed
13 Ar/Ap+36 AP	13 Ap+36 AP	98	All bivalents	Yes
13 Ar/Ap+18 AR	13 Ap+36 AP	80	13 bivalents+18 trivalents (similar to F1 hybrids)	–
13 Ar/Ap+ 9 AR +18 AP	13 Ap+36 AP	89	31 bivalents+9 trivalents	Yes

Ar,AR – *Roylei* chromosomes (bivalents and trivalents, respectively)
AP,Ap – *Pernyi* chromosomes (bivalents and trivalents, respectively)

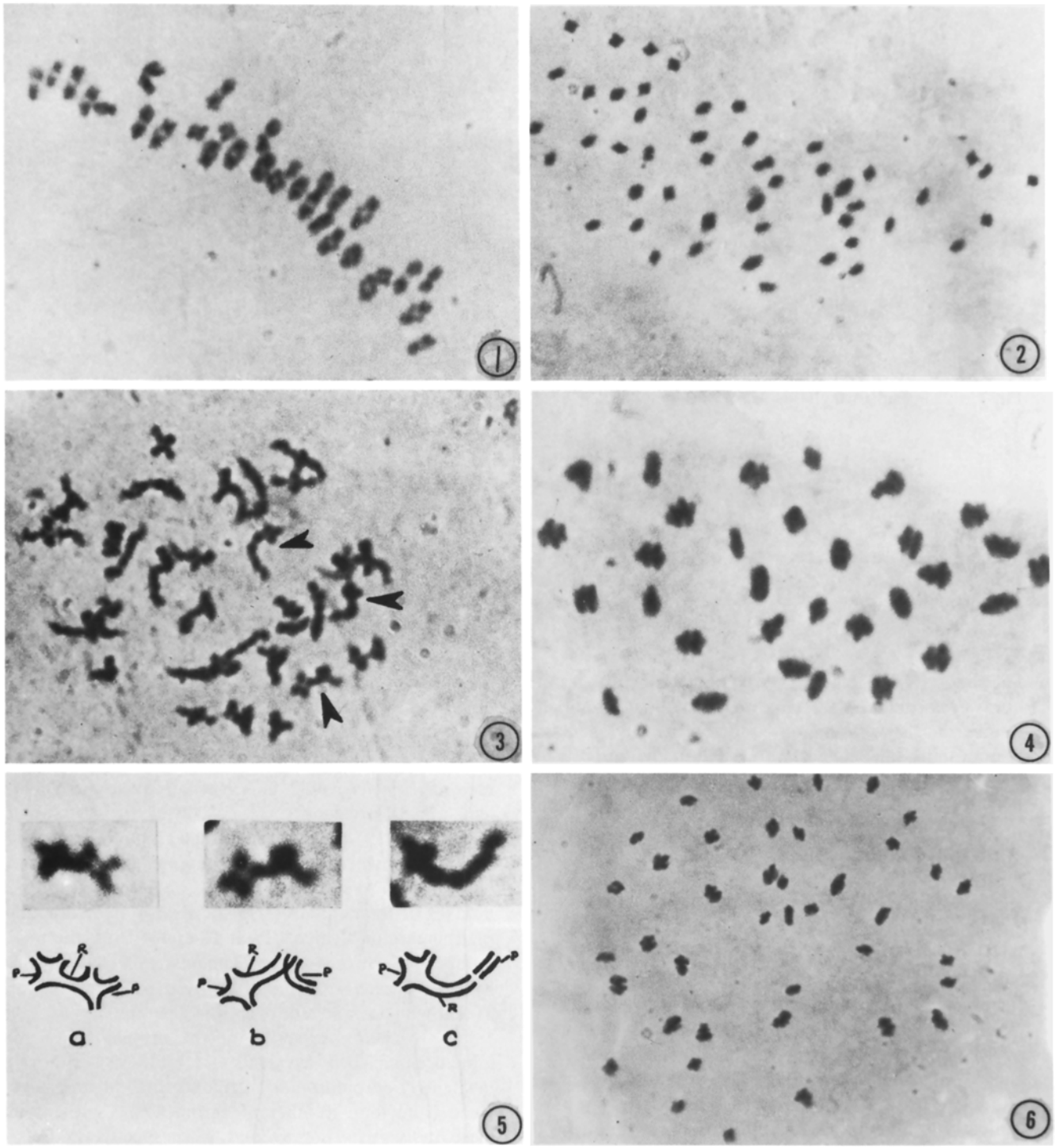

Fig. 1. *Antheraea roylei:* metaphase I plate with 31 bivalents

Fig. 2. *Antheraea pernyi:* metaphase I plate with 49 bivalents

Fig. 3. F1 hybrid: diplotene (*arrows* indicate trivalents)

Fig. 4. F1 hybrid: metaphase I with 18 trivalents and 13 bivalents

Fig. 5. F1 hybrid: enlarged trivalents showing localization of chiasmata

Fig. 6. Backcross individual: metaphase I with a trivalents and 31 bivalents

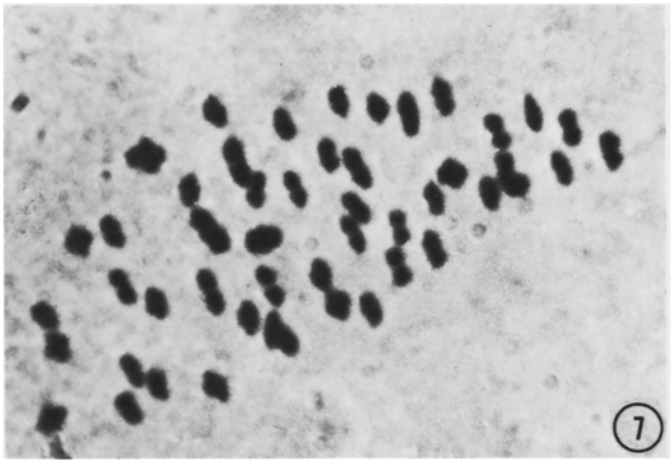

Fig. 7. F23 hybrid: metaphase I with 49 bivalents

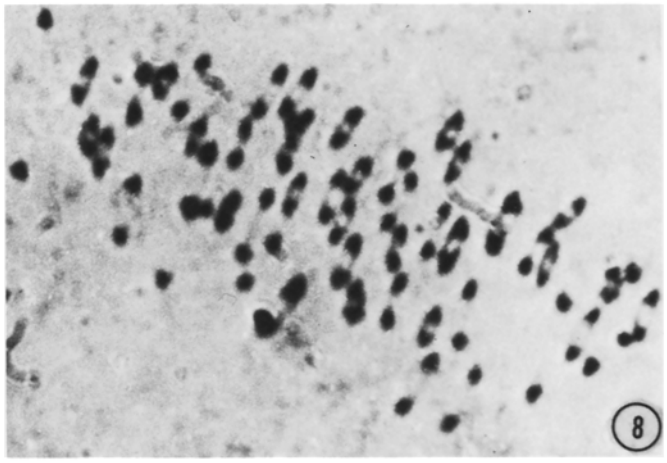

Fig. 8. F23 hybrid: early anaphase I

showed bivalents in Metaphase I (Fig. 7) and their segregation during Anaphase I was found to be normal (Fig. 8).

Discussion

Speciation in the order Lepidoptera is frequently, if not always, associated with chromosomal repatterning in number and structure. Consequently, the phylogenetic relationships can often be correlated at the karyotypic level by examining interspecific hybrids. When variation in chromosome number is accomplished by the process of fission, it is observed in hybrids in the form of trivalents or quadrivalents (Kawaguchi 1928; Federley 1939). A similar situation prevails in the hybrids used in the present investigation. Since F_1 hybrids showed a complement of 18 trivalents and 13 bivalents, the total number of chromosomes present is 80, of which 31 came from *A. roylei* and 49 from *A. pernyi.* Each trivalent is formed by the pairing of two *A. pernyi* chromosomes to one *A. roylei* chromosome and their association is retained by two chiasmata. Therefore, it can be inferred that 18 *A. roylei* chromosomes have undergone fission to give rise to 36 *A. pernyi* chromosomes. The remaining 13 chromosomes of *A. roylei* have been retained as such in *A. pernyi.* Regular meiotic pairing with chiasma formation and the normal reproductive vigour of F_1 hybrids which made possible the successful rearing of inbred offspring up to the F_{32} generation indicate that (1) the chromosome complements of *A. roylei* and *A. pernyi* share a high degree of homology and (2) even though the trend of karyotypic evolution from *A. roylei* to *A. pernyi* is towards a numerical increase, in about 60% of the chromosome complement of the former the genetic affinity between the two species is high despite their allopatry.

Jolly et al. (1969) obtained similar hybrids as those in the present study except that the *A. roylei* parent used then belonged to a different race (n=30). Theoretically, the hybrids obtained by Jolly et al. (1979) possessed 79 chromosomes of which 30 came from *A. roylei* and 49 from *A. pernyi.* In light of the observations made in the hybrid *A. roylei* (n=31)×*A. pernyi* (n=49), it could be explained that the 30 elements constituted 11 bivalents and 19 trivalents. This explanation could be further strengthened if we assume that the n=31 race of *A. roylei* used in this investigation is derived from the n=30 race by the fission of a chromosome.

On this basis the hybrid obtained from the n=31 race of *A. roylei* would be expected to show 13 bivalents and 18 trivalents since a *A. roylei* chromosome in one of the 19 trivalents mentioned above would be broken into two, thereby converting this trivalent into 2 bivalents. As a result, there would be a decrease in the number of trivalents from 19 to 18 with a corresponding increase in bivalents from 11 to 13. Thus, the total number of chromosomal elements expected in the hybrid with a n=31 race of *A. roylei* parent would be 31 and with a n=30 race of *A. roylei* parent, 30.

The backcross offspring of the present study exhibited either 40 (9 trivalents+31 bivalents) or 49 (all bivalents) chromosomal elements in Metaphase I. Hence, the total number of chromosomes present in these individuals are 89 and 98, respectively. These karyotypes can be observed in Table 1.

It is obvious from the table that backcross offspring with 80 chromosomal elements similar to F_1 hybrids were not observed by us. It is possible that we have not examined a sufficient number of backcross offspring to come across this type.

Meiotic analysis of F_{23} and F_{32} males derived from the cross effected by Jolly et al. (1969) between *A. roylei* (n=30) and *A. pernyi* (n=49) showed only 49 bivalents. The mor-

phology of the chromosomes of these individuals was very similar to that of the *A. pernyi* parent. Apparently all the chromosomes of *A. roylei* which were involved in the formation of trivalents are eliminated from the inbred hybrid progenies at some time. It is hazardous to venture any reason for the preferential elimination of these *A. roylei* chromosomes in the absence of karyotypic data for all the hybrid progenies. The only scanty data which are available come from the earlier work of Jolly et al. (1979) on F_2 individuals. While no definite trend is discernible from this information, there is some indication that a high percentage of F_2 offspring possessed a larger number of chromosomal elements than the F_1 hybrid. This is indicative of an increasing number of bivalents at the expense of trivalents. This in turn reflects a progressive but gradual elimination of *roylei* chromosomes of trivalents in succeeding generations of inbred hybrids.

At this juncture it can only be surmised that the presence of *roylei* chromosomes in question somehow lowers the relative fitness of the introgressed karyotypes and, therefore, they are selected against. This possibly may be related to favouring bivalent formation over trivalent formation by selection since the latter will definitely place a strain on the meiotic manoeuvre. Similarly, a karyotype with a high chromosome number simulating the *A. pernyi* parent got established in the later generations of inbred hybrids.

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