Some Cytological Observations on Interspecific Hybrid of *A. pernyi* G.M. and *A. roylei* Mr. (Saturniidae)

M. S. Jolly, S. K. Sen, G. K. Prasad and A. K. Sengupta

Central Tasar Research Station, Ranchi, India

Received August 1, 1977

Very few cases of successful introgressive hybridisation is on record in animal kingdom. Cousin (1934) obtained fertile progenies in the crosses between *Gryllus bimaculatus* ($2n=29$) and *G. campestris* ($2n=29$). Sturtevant (1920) reported that *Drosophila melanogaster* ($2n=8$) $\times$ *D. simulans* ($2n=8$) produced only fertile females, while the reciprocal cross resulted in fertile males. Mainland (1942) described that *D. limpiensis* ($2n=12$) $\times$ *D. macrospina* ($2n=12$) produced fertile females but the males were sterile.

In all these cases the difference in parental chromosome number was nil. Jolly *et al.* (1969), on the contrary, obtained fully fertile hybrids of two oak feeding tasar silkworm species, *Antheraea pernyi* (Chinese) and *A. roylei* (Indian), inspite of great disparity in their chromosome number (*A. roylei*: $n=30$, Jolly *et al.* 1970a, *A. pernyi*: $n=49$, Jolly *et al.* 1970b). The new hybrid has already crossed 20th generation successfully and laid the foundation of oak oriented tasar culture (Temperate tasar) in India (Jolly 1970). This perhaps is the only example of its kind in the insect world wherein the interspecific hybrid line could be maintained in the descending generations without registering any significant splitting of the different characters of economic interest.

The present paper records some cytological observations of this cross combination and explains the probable mechanism of chromosome compatibility between the two species resulting in fertile progenies.

Materials and methods

A. roylei Mr. and *A. pernyi* G.M. collected respectively from Jammu and Kashmir (India) and Japan served as materials for the present study. Both being high altitude oak feeding species, were maintained at J and K between altitudes 1200-1800 m AMSL on *Quercus incana*. The crosses *A. pernyi* $\times$ *A. roylei* and *A. roylei* $\times$ *A. pernyi* as also the back cross *A. pernyi*. *A. roylei* $\times$ *A. pernyi* (BC_1) were prepared and raised.

Testes from healthy male larvae (8th day after fourth moult) or from male pupae 20-25 days before metamorphosis were fixed in 1:3 acetic-alcohol for 4 hr and then preserved in 70 percent alcohol. The fixed materials were subjected to a thermic treatment of 50°C in aceto-carmin for an hour with a pinch of ferric chloride. Temporary squashes were prepared and the slides examined at a magnification of $16 \times 100X$. Camera lucida diagrams were drawn and microphotographs

taken wherever possible.

Observations

The frequencies of different chromosome numbers at F_1 , F_2 , and BC_1 , as revealed by the polar views of first meiotic metaphase, are presented in Table 1.

Table 1. Frequencies of different chromosome numbers at F_1 , F_2 and BC_1 in the crosses between *A. pernyi* and *A. roylei*

Sl. no.	Crosses	Generation	Individuals studied	Chromosome numbers								
				30	32	34	42	44	46	47	48	49
1.	<i>A. pernyi</i> × <i>A. roylei</i>	F_1	12	12	—	—	—	—	—	—	—	—
		F_2	15	—	4	—	4	6	—	1	—	—
2.	<i>A. roylei</i> × <i>A. pernyi</i>	F_1	4	4	—	—	—	—	—	—	—	—
		F_2	12	—	—	—	3	5	—	2	2	—
3.	<i>A. pernyi</i> × <i>A. roylei</i> × <i>A. pernyi</i>	BC_1	16	—	—	2	8	—	4	—	—	2

Fig. 1. The chromosomes at metaphase I in spermatocytes of F_1 , F_2 and BC_1 from the cross of *A. pernyi*, and *A. roylei*.

The interesting revelation of the study is the occurrence of only 30 chromosomes in the F_1 progenies, while the F_2 and BC_1 individuals showed varying numbers between 34 to 49 chromosomes.

Discussion

Introgressive hybridisation has been exploited considerably for evolving economically viable high yielders by mixing interspecific gene pools, thereby inducing the superior characters of one species to another. The interspecific hybrids have a definite field of usefulness provided the parental species have harmonious gene patterns. But the difficulties arise when the isolating mechanisms are absolute restricting the exchange of gene pools.

Table 2. Gain over mid-parent value in the cross
between *A. pernyi* × *A. roylei*

Sl. no.	Species	Cocoon weight (g)	Shell weight (g)	Reelable silk ratio (%)	Filament length (mtr)	Denier
1.	<i>A. pernyi</i>	4.6	0.41	8.90	411	5
2.	<i>A. roylei</i>	5.0	0.25	5.00	360	5
3.	M.P.V.	4.8	0.33	6.95	385	5
4.	F ₁ (<i>A. pernyi</i> × <i>A. roylei</i> and reciprocal)	6.5	0.70	13.84	748	5
5.	Gain over M.P.V. (%)	35.50	112.10	99.13	94.00	—

Fig. 2. Cocoons of (a) *A. roylei* (b) *A. pernyi* and (c) their hybrid.

The crosses between *A. roylei* and *A. pernyi* have yielded fertile progenies registering heterosis for all the important commercial characters (Table 2 and Fig. 2). The highest gain was observed in the characters, like shell weight (112.10%), reelable silk ratio (99.13%) and filament length (94.0%). Cocoon

weight, which is an index for measuring the vigour of the progenies, has also recorded considerable gain in the hybrid over the parents. However, the exact genetic mechanism resulting in such a higher degree of heterosis is not yet known.

Whatever may be the factors involved, the heterosis is due to the accumulation and interaction of certain advantageous genes. The level of heterosis further reflects that these two species have something very common in their ancestry, which still permits exchange of the gene pools between them.

The cytological analysis of the first filial generation of both cross and reciprocal showed only 30 chromosomal units reflecting thereby that 49 chromosomes of *A. pernyi* paired with 30 chromosomes of *A. roylei* and arranged themselves at the metaphase I plate in such a way as to give a polar view of 30 units (Fig. 3). Therefore in this case the gametes of the F_1 progenies would carry any number of chromosomes ranging from 30 to 49. The F_2 s of the cross and reciprocal revealing 32, 42, 44, 47 and 48 chromosome units and the back cross progenies exhibiting 34, 42, 46 and 49 units at metaphase I corroborate our contention.

Fig. 3. Diagrammatic presentation of the chromosome pairing in the cross between *A. pernyi* and *A. roylei*.

The chromosomal compatibility of the two species and hybrid fertility suggest that *A. pernyi* has possibly evolved from *A. roylei* through chromosomal dissociation. In certain genera of lepidoptera there is an extremely wide range of chromosome numbers e.g., from $n=8$ to $n=40$ in closely related species of *Erebia* and from $n=23$ to $n=191$ (the highest known chromosome number in animals) in *Lysandra nivescens* (White 1957). The explanation based on "dissociations" will suffice to explain these cases (White 1946). Lorkovic (1949) has suggested that the increasing number of chromosomes in Lepidoptera can be arranged in series and since they have diffused type of centromere the broken parts are not lost but behave as "new chromosomes". Recent cytological studies on *A. roylei* strengthens further the view that *A. pernyi* has evolved from *A. roylei* through chromosomal fission since the intraspecific chromosomal variants in the latter species like $n=30$, 31, 32 and 34 are frequent in the natural populations of north-eastern zone of India (Jolly *et al.* 1977). The two oak feeding species, which were involved in this study, have been conspecific in the past and are now largely allopatric due to establishment of geographical barriers.

References

- Cousin, G. 1934. Sur la fecondite normale et les caracteres des hybrides issus du croisement de deux especes de Gryllides (*Achaeta campestris* L. et *A. bimaculata* de Geer). C.R. Acad. Sci., Paris, **198**: 853-855.
- Jolly, M. S., Narasimbanna, M. N., Sinha, S. S. and Sen, S. K. 1969. Interspecific hybridisa-

- tion in *Antheraea*. Ind. J. Heredity **1**: 1-4.
- 1970. Oak oriented tasar culture in the offing. Indian Silk **8**: 3-5.
- , Sen, S. K. and Sinha, S. S. 1970a. Chromosome number in the Indian oak feeding tasar silkworm *A. roylei* Mr. Curr. Sci. **39**: 423-424.
- , — and — 1970b. Chromosome number in Chinese tasar silkworm, *Antheraea pernyi* G.M. (Saturniidae: Lepidoptera). Ind. J. Ent. **32**: 91-92.
- , —, Prasad, G. K. and Sahai, V. 1977b. Numerical chromosome polymorphism in *Antheraea roylei* Moore (Lep: Saturniidae). Cytology and Genetics In Press.
- Lorkovic, Z. 1949. Chromosomenzahlen-Vervielfachung bei Schmetterlingen und ein neuer Fall Fünfacher. Zähl. Rev. Suisse, Zool. **56**: 243-249.
- Mainland, G. B. 1942. Genetic relationships in the *Drosophila funebris* group. Univ. Texas Publication, **4228**: 74-112.
- Sturtevant, A. H. 1920. Genetic studies on *Drosophila simulans*. Genetics **5**: 488-500.
- White, M. J. D. 1946. The evidence against polyploidy in sexually reproducing animals. Am. Naturalist **80**: 610-619.
- 1957. Cytogenetics and Systematic Entomology. In Annual Review of Entomology (E. A. Steinhaus & R. F. Smith ed.), **2**: Entomological Society of America 71-90.
-