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CHROMOSOMAL ABERRATIONS IN RHINOCEROS BEETLE (*ORYCTES RHINOCEROS*) INFECTED WITH BACULOVIRUS

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ABSTRACT

Chromosomal aberrations in the testicular and ovarian cells of rhinoceros beetle infected with baculovirus in vivo were studied. Chromosomal/chromatid breakage (female 32.2%, male 28.2%) and centromeric dissociation (female 29.6%, male 24.0%) were more frequent among the aberrations followed by aneuploidy (female 14.8%, male 6.3%), acentric (female 11.6%, male 4.5%) and centric (female 2.8%, male 1.3%) fragments. Metacentrics were 1.6% and 1.2%, respectively, in the female and male cells. Females were more susceptible to the virus infection in terms of chromosomal aberrations compared to male beetles, the total frequency of aberrant cells being 65.5% in males and 92.6% in females. The mean number of abnormalities per aberrant cell was 1.01 in both male and female.

Key words: Rhinoceros beetle, chromosomal aberrations, baculovirus.

Rhinoceros beetle, *Oryctes rhinoceros* L., (Coleoptera : Dynastinae) is a serious pest of coconut palms [1–3] and the incidence of a baculovirus as a pathogenic organism of rhinoceros beetle has been recorded in Malaysia [4] and Kerala (India) [5]. Efficacious use of baculovirus as a biological control agent for rhinoceros beetle has been reported [6–12]. Chromosomal distortions in animal cells due to microbial infection in vivo were observed by many investigators [13–26]. Though the karyotype of the rhinoceros beetle has been reported [27–29], no attempt was made to study the interaction of the virus at chromosomal level in the beetle. The present paper reports the effect of baculovirus infection on the chromosome of rhinoceros beetle.

MATERIALS AND METHODS

Adult rhinoceros beetles were reared in the laboratory on chopped coconut fronds. Twenty five beetles (both males and females)were inoculated with baculovirus following the method of Mohan et al. [5] and the manifestation of disease among the beetles was confirmed. Testes and ovaries (from both healthy and diseased beetles) were dissected out from the beetles anaesthetized with ether and kept in normal physiological saline.

The tissues were treated with aqueous solution of sodium citrate (1%, w/v) for 30 minutes. The tissues were macerated in acetomethanol (1:3 v/v) and washed twice in the same solution by thorough mixing, discarding the supernatant each time by decantation. The cells were treated with the Hungerford fluid [30] for 7 minutes before the slides were prepared by air drying method under hot air [31]. The slides were treated with aceto-methanol (1:3 v/v) once again, washed in distilled water and stained with Giemsa prepared in phosphate buffer (pH 6.8). Observations were recorded at metaphase I.

RESULTS AND DISCUSSION

Chromosomal aberrations were observed both in male and female sex cells, though each sex responded differently. The aberrations were more preponderant in the ovarian cells than in testes (Table 1). Chromosomal breaks (female 32.2%, male 28.2%) and centromeric dissociations (female 29.6%, male 24.0%) were more frequent among the different types of aberrations. Chromatid/chromosomal breaks were evident in the distal regions of the metacentric (Fig. 1: 3, 5) and submetacentric (Fig. 1: 4) chromosomes. The chromosomes were vulnerable to breakages due to the presence of some weaker sites [18] or immunogenetic background [32]. The viral interaction with the host cells was a causative factor for chromosomal damage characterized by the physicochemical nature of chromosomes in the course of DNA synthesis or repair [13–21, 25]. The incidence of aneuploidy (female 14.8%, male 6.3%, Fig. 1: 2–4], acentric (female 11.6%, male 4.5%, Fig. 1: 3, 4) and centric (female 2.8%, male 1.3%, Fig. 1: 4) fragments were also observed in some cases. Acentric and centric fragments of different sizes were observed which might be functional or nonfunctional depending on the situation. Centromeric dissociations

Table 1. Chromosomal aberrations in the meiotic cells of male and female rhinoceros beetle infected with baculovirus

Chromosomal aberration	Frequency (%)	
	ovary	testis
Centromeric dissociation	29.6	24.0
Chromatid/chromosomal breakage	32.2	28.2
Aneuploidy	14.8	6.3
Acentric fragments	11.6	4.5
Centric fragments	2.8	1.3
Metacentrics	1.6	1.2
Normal	7.4	34.5
Total No. of metaphases	360	340

Table 2. Frequency of chromosomal abnormalities in the gonadal tissues of male and female rhinoceros beetles infected with baculovirus

Parameter	Male	Female
No. of metaphase	340	360
Total aberrant cells (%)	65.5	92.6
No. of chromosomal abnormalities per aberrant cell	1.01	1.01

were not restricted to any particular chromosome, they could be seen practically in all chromosomes (Fig. 1: 2–4). Similarly, addition or deletion of different chromosomes were noticed in the case of aneuploidy (Fig. 1: 2, 3). A few cases of metacentrics (1.6% in female

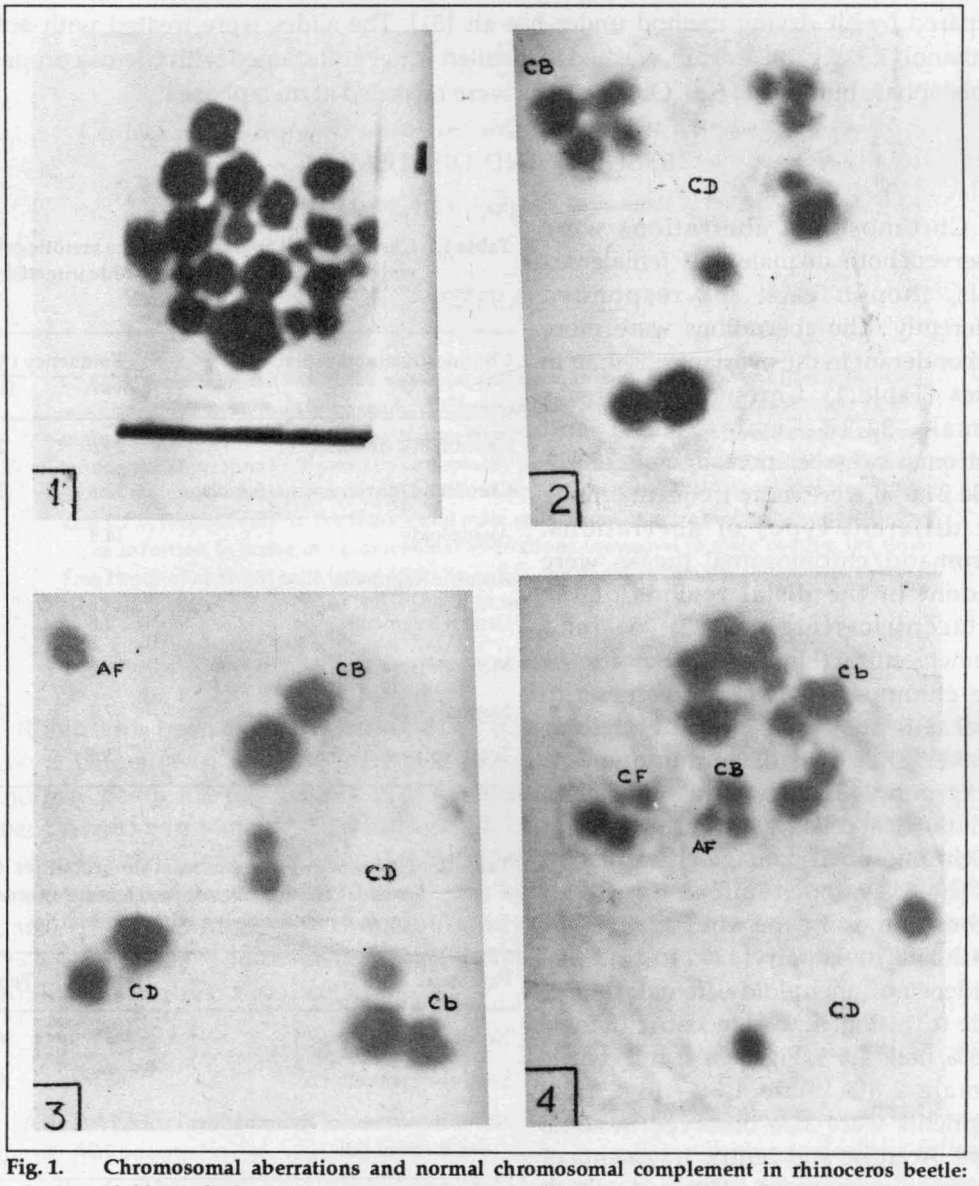

Fig. 1. **Chromosomal aberrations and normal chromosomal complement in rhinoceros beetle: 1) Normal chromosomal complement. 2–4) Aneuploids. CB—chromosomal breaks, cb—chromatid breaks, CD—centromeric dissociation, AF—acentric fragments, and CF—centric fragments.**

and 1.2% in male) were also recorded. The number of aberrant cells was high in female (92.6%) than in male (65.5%) (Table 2). However, the average number of chromosomal abnormalities per aberrant cell was found same (1.01) in both the cases.

The manifestation of chromosomal aberrations, similar to cancerous type of cells, and rearrangement of chromosomes would affect the physiological processes including development of normal cells in rhinoceros beetle infested with baculovirus and also the development of beetle progenies. It is evident that the chromosomal set-up of the beetle is irreversibly damaged due to the viral infection. The present study thus supports Caltagirone's reference to the virus as a successful biocontrol agent for the rhinoceros beetle in molecular terms [7].

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