Original Article

Effects of Different Feeds on the Chromosome Behaviour of *Zonocerus variegatus*

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Submitted: June 12, 2012; Accepted: Nov. 12, 2012; Published: Dec. 17, 2012.

ABSTRACT

The effects of different feeds (*Aspilia Africana*, *Manihot utilissimma*, *Musa paradisca*, *Costus afer*, *Chromolaena odoratum*) on the chromosomal behaviour of *Zonocerus variegatus* were studied. The means (17.0 $\pm$ 0.20) for chiasma frequency was highest in the insects fed with a wide variety of feeds or Control, and this was followed by *Manihot utilissimma* fed insects (16.25 $\pm$ 0.28).This was significantly different from *M. paradisca* and *C. afar* ($p<0.01$) and also from *C. odoratum* ($p<0.1$). The lowest mean for chiasma frequency was recorded from the insects raised on *C. odoratum* (14.65 $\pm$ 0.85) and *C. afar* (14.0 $\pm$ 0.91). These two feeds induced more frequent chromosomal aberrations which included non-pairing of bivalents, multivalent associations, lagging chromosomes, dicentric bridges and late separations.

Keywords: *Zonocerus variegatus*, Chromosomal aberration, Chiasma frequency

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INTRODUCTION

Zonocerus variegatus is a chronic but, in general, a relatively minor pest (COPR, 1977). Locally, however, the population can be very large and cause considerable defoliation and subsequent crop yield loss. *Z. variegatus* causes damage to economic cash and food crops by reducing the photosynthetic area, which results in yield reductions, and by attacking stems, which weakens the plant, inhibits nutrient transport and exposes the plant to viral and bacterial diseases.

Locusts and grasshoppers can also destroy food sources for many animals and thus affect biodiversity; such effects may be particularly pronounced in isolated insular ecosystems (Latchininsky, 2008). Large-scale locusts and grasshoppers control programs can also affect biodiversity, including that of nontarget grasshoppers (Samways, 2000).

This study was undertaken to examine the effects of selected feeding on the reproductive capacity (that is, meiotic process) of *Z. variegatus*. Such understanding could help in the development of biological measures for the control of this pest.

MATERIALS AND METHODS

Group Feeding Experiments

Five cages were used for the feeding experiment. Each cage had a 48 x 45.5 x 45.5cm dimension and was covered on three sides by wire gauze. The remaining sides, the top, bottom and one vertical side, were covered with plywood. The top had a lid on it. A sixth cage was used for the storage of nymphs collected from the wild and had a dimension of 63x45.5 x 45.5cm.

The cages were large enough to provide maximum activity for the insects, and the gauze gave enough ventilation and light inside the cages. One hundred 1st & 2nd instar nymphs were placed in the five treatment cages and fed as follows: cage 1- *Aspilia africana*, Cage 2- *Manihot utilissima*, Cage 3- *Musa paradisca*, Cage 4-*Costus afer* and cage 5- *Chromolaena odoratum*. All cages were placed in the open in order to subject them to normal fluctuations of temperature and humidity. Adults of the *Z. variegatus* were collected from the wild as control.

The Feeds

Fresh leaves of *Aspilia africana, Chromolaena odoratum, Costus afer, Musa paradisca* and *Manihot utilissima* were collected from the field in the evenings between 5.30pm and 6.00pm. The leaves were placed inside cages on a water container with the basal parts of the leaves (petiole) immersed in water to minimize desiccation of the feeds.

Killing, Dissection, Extraction and Preservation of Testes

Cotton wool was soaked in Chloroform and applied on the adult (mature) insects for 2-3mins to kill them. For dissection, the adult male was pinned with the ventral side facing down in the middle of a dissecting dish containing water, with the aid of a pair of sharp scissors and a pair of forceps, a longitudinal cut was made along the mid-dorsal line from the neck region down to the last segment of the abdomen.

With the help of the pair of forceps, the whole of the alimentary canal was removed and this helped to expose more conspicuously the testis which is made up of many testicular follicles. The testes were removed and stored in sample bottles containing 1:3 acetic alcohol. The sample bottles were labeled according to the vegetation fed on the insects whose testes were extracted, and were preserved in the refrigerator for about 12-24 hours before squashing of the testes was carried out.

Squashing of Testes

Before the squash preparation, the slides and cover slips were thoroughly cleaned with absolute alcohol. A few (1-4) tubules of the preserved testes were separated out with the aid of a pair of forceps and a needle. A drop of aceto carmine (a basic dye used to stain the chromosome) was placed on the clean slide, tubules were then smeared with the needle and a cover slip was placed on the smear.

The slide was then warmed gently over a spirit lamp flame. This procedure helped in flattening the cells, spreading the chromosomes and making them stick on the slide and cover slip. The slide was then placed within the fold of a filter paper and a gentle thumb pressure applied on it. To prevent entry of air into prepared slide, colourless nail varnish was applied around the edges of the cover slip.

Cytological Observations

The slides were mounted individually on the microscope and the sperm mother cells observed first under X100 and later under X400 magnifications. At X400 magnification the chiasma positions in bivalents were conspicuous especially at diakinesis and metaphase I stages. The cells at diakinesis and metaphase I stages were drawn and their chiasma frequency determined for twenty sperm mother cells (SMC'S) from each of the eight insects selected from the different feeds. Twenty (20) SMC's was scored and their chiasma frequencies determined.

Preparation of Perment Slides

Nine glass Petri dishes each with two short glass rods were placed in series. A solution of acetic alcohol was added to the first Petri dish, followed by Petri dishes containing 50%, 70%, 90% and absolute alcohol respectively. The sixth Petri-dish contained 50% Xylene and 50% alcohol. The proportion of Xylene was gradually increased to 70%, 90% and 100% in the last three Petri-dishes.

The slides to be made permanent were first inverted into the solution of acetic alcohol in the first Petri-dish with one end raised up with the help of two glass rods. This was left

until the cover slip slipped off from the slide. The slide and the cover slip were then passed through the alcohol and Zylene series in the Petri dishes. Each slide and cover slip was allowed about two minutes in each Petri-dish. Finally after two minutes at the last Petri-dish containing 100% Xylene, the slides and the corresponding cover slips were taken out and a drop of Canada balsam added to the slide and the cover slip replaced on it. This was later left in an oven to dry at 65º C for two to three days.

RESULTS AND DISCUSSION

The result of **survival rate** of *Z. variegatus* on different feeds showed that it depended on the food plant provided, and developmental stages (Table 1). *C. afer* gave the highest death rates in the first and second instar stages. The reason for the high death rate appears to be due to the fact that the leaf was very thick and hard for the second and third instar nymphs to feed on. The deaths of the insects were therefore attributed to starvation. After replacing the third instars with the fourth instars, the survival rate increased to 68%. This sudden high survival rate was apparently attributable to a more developed and better adapted mouth parts (mandibles and maxillae), digestive caeca and age. Also correlations between feeding habits and structures of the mandibles and maxillae, tarsal arolia and digestive caeca were noted by Kaufmann (1965).

Result showed that the control insects which were not fed in captivity had the highest mean for chiasma frequency. This was followed by *M. utilissima*-fed > *A. africana* fed >*C. odoratum*- fed > *C. afer* > *M. Paradisca* -fed respectively. It was observed that chromosomal aberrations occurred more frequently in the *C. afer* and *Chromolaena* fed insects. In the diakinesis, such abnormalities as non-pairing of bivalents and multivalent associations were noted. In Metaphase 1, there were observed high frequencies of precocious disjunction and non-pairing of bivalents. (Plate 3) In Anaphase 1, there were also noted lagging chromosome, dicentric bridges and late separation (Plates 1 and 2). In agreement with this study, Lasebikan and Olorode (1972) working on morphological variations and chromosome aberrations in *Z. variegatus* reported folded segments and chromatin connections in early diplotene stage. In the Anaphase 1, they also reported non-disjunction of chromosomes.

According to the FAO (2004) biological control is a component of the Integrated Pest Management (IPM) system which was developed in response to overdependernce on pesticides.

CONCLUSION

Z. variegatus fed with different feed types in captivity is selective in feeding. The study shows that different feeds induce varied frequencies of the Chiasma. Some of the feeds induced more chromosomal aberrations than others. There is indication that it is possible to induce chromosomal aberrations in Z. variegatus using preferential feed. This work is a prelude to future successful biological control for Z. variegatus.

Table 1: Percentage Survival under Different Feeds

	Survival (%) of instars with feeds				
INSTARS	*M. utilissima*	*A. africana*	*C. odoratum*	*M. paradisca*	*C. afer*
2nd	90	77	46	74	03
3rd	97	96	57	75	01
4th	90	87	67	76	68
5th	87	100	76	87	55

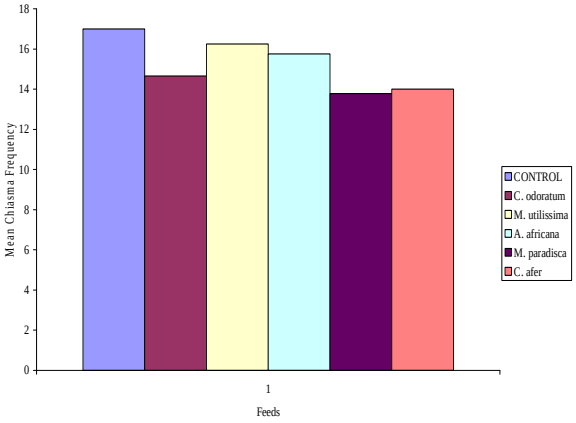

Fig. 1: Mean Chiasma Frequency observed with Z. variegatus with different feeds and a control

Table 2: Means of Chiasma frequency of *Z variagatus* for different feeds

FEEDS	NO.	MEAN	CONFIDENCE LIMIT (95%)
Chromolaeana odoratum	8	14.65	± 0.85
Manihot utilissima	8	16.25	± 0.28
Aspilia Africana	8	15.76	± 0.84
Costus afer	8	14.0	± 0.91
Musa paradisca	8	13.79	± 1.31
Control	8	17.00	± 0.20

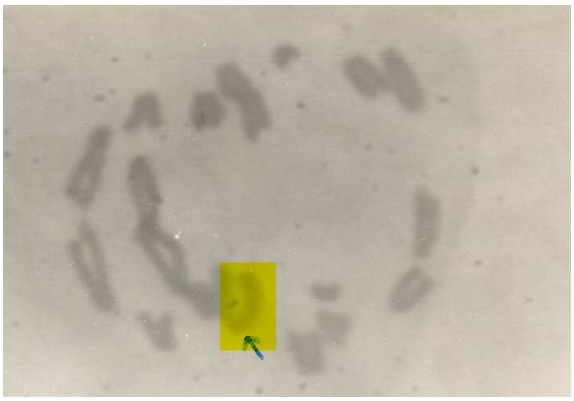

Plate i: Anaphase 1 bridge in male *Z. veriagatus*. Highlited area shows the sex (X) Chromosome

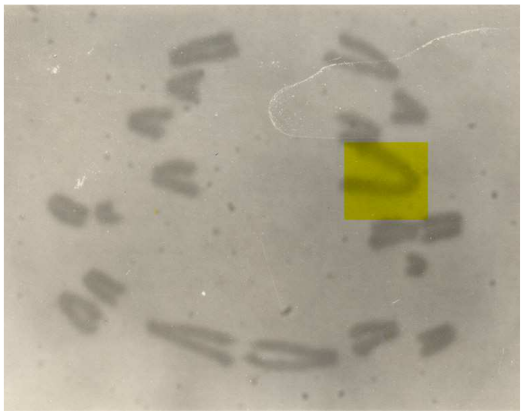

Plate ii: Anaphase 1 Late seperation in male *Z. veriagatus*

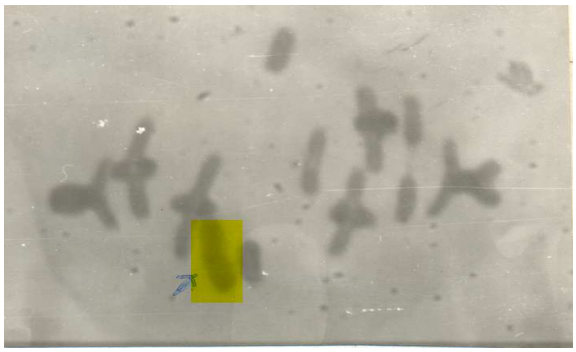

Plate iii: Metaphase 1 with non-pairing of bivalents. Highlited area shows the sex (X) Chromosome

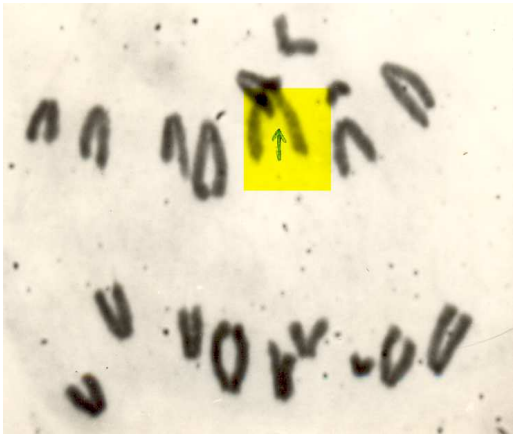

Plate iv: Mid Anaphase 1 of *Z. variagatus*. Highlited area shows the sex (X) Chromosome

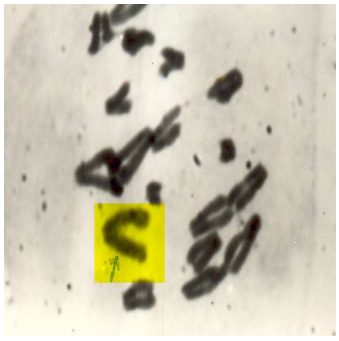

Plate v: Late Metaphase 1 in *Z. variegatus.* Seperation difficulty. Highlited area shows the sex (X) Chromosome

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