INNSPUB

**International Journal of Biosciences | IJB |**
**ISSN: 2220-6655 (Print) 2222-5234 (Online)**
**http://www.innspub.net**
**Vol. 3, No. 4, p. 1-7, 2013**

RESEARCH PAPER OPEN ACCESS

# The effect of the dry and wet seasons on chiasma frequency in male *Taphronota thaelephora* Stal. 1873 and *Zonocerus variegatus* L. 1753 (Orthoptera: Pyrgomorphidae) in Cameroon

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**Key words:** Chiasma frequency, dry and wet seasons, *Taphronota thaelephora*, *Zonocerus variegatus*.

doi: http://dx.doi.org/10.12692/ijb/3.4.1-7 Article published on April 22, 2013

## Abstract

A study of the male meiotic systems in dry and wet season populations of the African pest grasshoppers *Taphronota thaelephora* and *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae) was carried out. Data obtained revealed that mean chiasma frequency was significantly higher in the dry than wet season populations of these species. However, Duncan Multiple Range Test revealed that mean chiasma frequency was not significantly different ($P>0.05$) between dry and wet season populations of *T. thaelephora* but was significantly higher ($P<0.05$) in the dry than wet season populations of *Z. variegatus*. Differences in the number of rod and ring shaped bivalents in Diplotene were not consistent in the dry and wet season populations of both species nor were the number of bivalents with 1, 2 and 3 chiasmata. These results indicated that this environmental cue is not responsible for the observed differences in chiasma frequency in these pest species.

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## Introduction

*Taphronota thaelephora* Stal. 1873 and *Zonocerus variegatus* L. 1753 are common grasshoppers in the Western Highlands of Cameroon, where their pest activities are well known (Nonveiller, 1984; Seino *et al*, 2001). In the West Region of Cameroon, adults of these species can be observed in the field throughout the year with peak populations densities between the months of November and February here referred to as the dry season population and between the months of March and August here referred to as the wet season population. However *T. thaelephora* does not gather in large voracious groups and is therefore a pest of minor agricultural consequence in this region of Cameroon compared to *Z. variegatus* known to inflict high damage to crops wherever it is found (Nonveiller, 1984; Iduwu and Modder, 1996). In the Humid Forest Zone of Cameroon, farmers perceive *Z. variegatus* as the third most important insect pest (Kekeunou *et al*, 2006). For some time now, both of these insects have been generating a lot of interest for biology and cytogenetics studies.

Much is known about the variation of chiasma frequency in Orthoptera grasshoppers and available data comes from both laboratory stocks and populations from the wild. It is a well known fact that chiasma frequency is regulated both genetically and by environmental components. A review of the investigations of White, (1973), Nolte *et al*, (1969), Oyidi (1967a, b); Shaw (1971) and Seino, (1989) suggests a response pattern with considerable variability in sensitivity to the environment. In these investigations, however, discrepancies in the response pattern obtained suggest considerable variability in sensitivity to the environment. Thus, not only do different systems of chiasma control respond differently to environmental fluctuations but within a species, the pattern of response is not constant between genotypes (Verma and Agarwal, 2005). While differences in chiasma frequency between individuals have long been considered to be adaptive (Gupta, 2005), it is also clear that different regions of the same genome can respond differentially. By implication, therefore, adaptive differences can arise within as well as between seasonal populations. Indeed, variation in the sensitivity of a genotype to short term environmental change such as the dry and wet seasons would provide an organism with a rapid means of adjusting its release of variation. This applies especially to the gene systems which govern chiasma formation since variation in their expression within an individual influences the range of variability in the succeeding generation.

Chiasma frequency variation has been shown to follow a definite annual cycle in reptiles (King and Hayman, 1978; Cobror *et al*, 1986). In insects, Oyiydi (1967b) observed chiasma frequency to be higher in dry than wet season populations of *Z. variegatus* in Nigeria due to marked reduction in terminal chiasmata. Seino (1989) reported chiasma frequency to be higher in the dry than wet season populations of *Morphacris fasciata* and *Coryphosima stenoptera producta* (Acrididae), but higher in the wet than dry season populations of *Chirista compta* and *Oxycatantops spissus* (Acrididae). Seino (1989) also observed that chiasma frequency was not different in the dry and wet season populations of *Acrida turrita*, *Paracenema luculenta* and *Spathosternum pygmaeum* (Acrididae). From the above reports it is clear that the manner in which the dry and wet seasons affect chiasma frequency in grasshoppers is still obscure. This investigation was therefore designed to confirm whether the dry and wet seasons affect chiasma frequency and to determine if the control of chiasma frequency in grasshoppers is a simple bivalent based system.

## Materials and methods

Two Cameroonian Pyrgomorphidae species, *T. thaelephora* and *Z. variegatus* were used for this study. These species were chosen because they are pest species, common to find, have adapted successfully to the Cameroonian environment (dry and wet seasons) and have the same chromosome number (2n= 19 ♂). (Williams and Ogunbiyi, 1995; Seino *et al.*, 2002), but are different in chiasma distribution (Oyidi; 1967a & b; Seino *et al.*, 2002).

These biological materials consisted of 40 adult individuals of *T. thaelephora* Stal. and *Z. Variegatus* L. The insects were collected from the wild during the dry season of December 2010 and wet season of June 2011. The grasshoppers were hand-picked from bitter leaf (*Vernonia amydalina*) and cassava (*Manihot esculenta*) plants in farms at Balepa - Mbouda in the West Region of Cameroon. On capture, the insects were immediately killed with chloroform fumes and dissected in insect saline (0.68% NaCl) for the testes. The testes obtained were then fixed in 3:1 ethanol: acetic acid fixative and stored in the refrigerator at 4°C until used.

*Meiotic chromosome analysis*

Meiotic chromosome smears were prepared using the Lactic-Propionic-Orcein squash technique of Seino *et al.*, (2010). Two to three testicular follicles were placed on a clean microscope glass slide. They were first flooded with 45% acetic acid for five minutes. This made the cells to swell. After blotting off the acid, the tissue was next flooded with one or two drops of Lactic-Propionic-Orcein stain and macerated using the sharp pointed end of a dissecting needle. This permitted the stain to penetrate into the tissue. The preparations were then incubated at room temperature between ten and fifteen minutes while making sure that the stain did not dry off. A cover slide was next placed on the tissue, held in place with the thumb and forefinger before gently tapping with the wooden end of a dissecting needle. This enabled the cells to disperse and force out excess stain. The preparation was then wrapped in a filter paper and squashed between the thumb and the top of the laboratory table. The filter paper absorbed excess stain. The edges of the cover slide were sealed with colourless nail vanish to temporarily preserve the preparation. A minimum of 200 cells were used for data collection.

Diplotene / Diakenesis chiasma frequency counts, the number of rod and ring bivalents and the frequency distribution of bivalents with 1, 2 and 3 chiasmata in five cells per individual were scored. Data collected was subjected to the ANOVA test, Duncan's Multiple Range Test (DMRT) and the Student's t – test to determined significant differences between species and seasons. All probabilities were appreciated at the 5% level of significance.

## Results and discussion

The events which take place prior to and during chiasma formation are regulated by both genetical and environmental components (Shaw, 1971). The genetic mechanisms involved are still not well understood. However, evidences abound to the fact that chiasma formation in many organisms can be modified by environmental factors (Verma and Agarwal, 2005). Information concerning natural variation in chiasma frequency can be a valuable tool to characterise phenotypic traits and their genetic control. Chiasma frequency also reflects aspects of the release of variability in sexually reproducing organisms (Lopez *et al.*, 2012). Chiasma frequency is known to provide a means of investigating components of the genetic systems of grasshoppers and other sexually reproducing organisms. It is a commonly held view that a species possesses a certain mean chiasma frequency consistent within normal sampling variation. However, different types of variation have been recorded in both plants and animals. There are accounts of inter-population variations (Sanchez-Muran *et al.*, 2002) with some of these variations being attributed to environmental factors such as the dry and wet seasons. We wish to now analyse the data collected for *T. thaelephora* and *Z. variegatus* in the dry and wet seasons.

The results obtained from sequential samples taken from 40 adult males of *Taphronota thaelephora* and *Zonocerus variegatus* are presented in Table 1. This table therefore contains chiasma frequency means in the dry and wet season populations of these two species. Chiasma frequency was normally distributed in both dry and wet season populations of the 2 species studied (Fig. 1 and 2). Table 1 also revealed that mean chiasma frequency was higher in the dry than wet season populations of the two species. However, the range was larger in *Z. variegatus* (1.12 chiasmata) than in *T. thaelephora* (1.0 chiasma). The data in Table 1 was subjected to ANOVA and the F value obtained (23.61) (Table 2) indicated that the mean chiasma frequency in the dry season was significantly higher (P<<0.05) than mean chiasma frequency in the wet season. Therefore, the dry season significantly induced the formation of chiasmata than did the wet season. The dry season is known for its harsh climatic conditions such as increased temperatures, unavailability of water and fresh leaves (food). Farming activity is reduced in the dry season further compounding the food problem of these pest grasshoppers. The need therefore arises for the grasshoppers to released increased variation through increased chiasma frequency so as to provide a means for adaptation. Chiasma frequency reflects on the aspect of the release of variability (King and Hayman, 1978). The data in Table one was also subjected to the Duncan Multiple Range Test (DMRT) to determine whether the dry and wet seasons affect chiasma frequency in the two species to the same magnitude. The test results revealed that there was no significant difference (P>0.05) in the mean chiasma frequency in the dry and wet seasons for T. *thaelephora* while the mean chiasma frequency in the dry season was significantly higher (P<0.05) than in the wet season for *Z. variegatus*. Therefore season affected chiasma frequency in *Z. variegatus* but not in *T. thaelephora*.

**Table 1.** Mean chiasma frequency in the dry and wet season populations of *T. thaelephora* and *Z. variegatus*. Five cells were considered from ten individuals per population.

| Species | Season | Individual | | | | | | | | | | Mean Chiasma Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| *T. thaelephora* | Dry | 11.6 | 11.2 | 11.6 | 13.6 | 12.6 | 12.8 | 12.0 | 12.8 | 12.6 | 12.8 | 12.36±0.69 |
| | Wet | 10.2 | 12.0 | 12.2 | 12.6 | 12.8 | 12.0 | 12.6 | 13.0 | 12.6 | 12.6 | 12.26±0.75 |
| *Z. variegatus* | Dry | 14.6 | 15.0 | 14.2 | 14.4 | 15.2 | 15.4 | 14.4 | 14 | 15.2 | 14.6 | 14.72±0.45 |
| | Wet | 12.6 | 15.4 | 13.6 | 13.6 | 14.0 | 13.2 | 12.6 | 12.6 | 14.8 | 13.6 | 13.60±0.89 |

**Table 2.** F ratios for the comparison of effects of dry and wet seasons on chiasma frequency in *T. thaelephora* and *Z. variegates*.

| Source | Type III Sum of Squares | df | Mean Square | F | Significance |
|---|---|---|---|---|---|
| Corrected model | 48.096 | 12 | 4.008 | 8.315 | 0.000 |
| Intercept | 7006.609 | 1 | 7006.609 | 1.454E4 | 0.000 |
| Replications | 7.581 | 9 | 0.842 | 1.747 | 0.126 |
| Season | 2.809 | 1 | 2.809 | 5.827 | 0.023 |
| Species | 34.225 | 1 | 34.225 | 71.001 | 0.000 |
| Season*species | 3.481 | 1 | 3.481 | 7.221 | 0.012 |
| Error | 13.015 | 27 | 0.482 | - | - |
| Total | 7067.720 | 40 | - | - | - |
| Corrected Total | 61.111 | 39 | - | - | - |

**Table 3.** Frequency of rod and ring bivalents, bivalents with 1, 2 and 3 chiasmata in dry and wet season populations of *T. thaelephora* and *Z. variegates*.

| Species | Season | Mean chiasma frequency | Rod shaped bivalents | | Ring shaped bivalents | | 1 - chiasmata | | 2 - chiasmata | | 3 – chiasmata | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | X | % | X | % | X | % | X | % | X | % |
| *T. thaelephora* | Dry | 12.36±0.69 | 6.28 | 69.78 | 2.72 | 30.22 | 6.28 | 69.78 | 2.18 | 24.22 | 0.54 | 6.00 |
| | Wet | 12.26±0.75 | 6.44 | 71.56 | 2.56 | 28.44 | 6.44 | 71.56 | 1.80 | 20.00 | 0.76 | 8.44 |
| *Z. variegatus* | Dry | 14.72±0.45 | 5.20 | 57.78 | 3.80 | 42.22 | 5.22 | 58.00 | 1.86 | 20.67 | 1.92 | 21.33 |
| | Wet | 13.60±0.89 | 5.76 | 64.00 | 3.24 | 36.00 | 5.76 | 64.00 | 1.88 | 20.89 | 1.36 | 15.11 |

We also assessed the frequencies of Diplotene rod and ring shaped bivalents, bivalents with 1, 2 and 3 chiasmata to find out if the dry and wet seasons affected the formation of these types of bivalents. Table 3 revealed that in both *T. thaelephora* and *Z. variegatus,* the frequencies of rod bivalents were higher in the wet than dry seasons while the converse was true for ring bivalents. Also the frequency of bivalents with 1 chiasma and 3 chiasmata were higher in the wet than dry season while the frequency of bivalents with 2 chiasmata were higher in the dry than wet seasons for *T. thaelephora*. similarly, the frequencies of bivalents with 1 chiasma was higher in the wet than dry season, the frequency of bivalents with 2 chiasmata were not different in the two seasons while the frequencies of bivalents with3 chiasmata were higher in the dry than wet season for *Z. variegatus*. There was therefore no consistency in the frequency of the different types of bivalents in the dry and wet seasons in the two species. Student's t-test values (Table 4) revealed that the frequency of rod and ring bivalents was not significantly different (P>0.05) in the dry and wet seasons for *T. thaelephora*. On the other hand, the frequency of rod bivalents was significantly higher in the wet than dry season while the frequency of ring bivalents was significantly higher in the dry than wet seasons in *Z. variegatus*. The frequencies of bivalents with 1 chiasma and 2 chiasmata were significantly different in the dry and wet season for *T. thaelephora* while the frequencies of bivalents with 1 chiasma and 3 chiasmata were significantly different in the dry and wet seasons for *Z. variegatus* (Table 4). Once more there was no consistency in the effect of the season in the two species.

**Table 4.** Student's t-test values calculated to compare the effect of the dry and wet seasons on formation of rod and ring bivalents and bivalents with 1, 2 and 3 chiasmata in *T. thaelephora* and *Z. variegates*.

| Type of bivalent | Student' s t-test for equal means calculated t | Levene's test for equality of variance | Significance |
|---|---|---|---|
| | *a) T. thaelephora* | | |
| Rod | 0.755 | 0.307 | 0.586 |
| Ring | 0.751 | 0.301 | 0.590 |
| 1 chiasma | 0.424 | 3.588 | 0.074 |
| 2 chiasmata | 2.204 | 1.714 | 0.207 |
| 3 chiasmata | -1.971 | 0.069 | 0.796 |
| | *b) Z. variegatus* | | |
| Rod | -2.745 | 2.225 | 0.013 |
| Ring | -2.745 | 2.225 | 0.013 |
| 1 chiasma | -3.533 | 0.077 | 0.002 |
| 2 chiasmata | -0.081 | 8.728 | 0.937 |
| 3 chiasmata | 3.175 | 13.819 | 0.005 |

It can be concluded from above evidences that the formation of rod and ring bivalents, as well as bivalents with 1, 2 and 3 chiasmata were not affected by the dry and wet seasons for the two species. Therefore this environmental cue is not responsible for the cyclic nature of chiasma frequency in *T. thaelephora* and *Z. variegatus* collected in Cameroon.

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