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## Cytological perspectives of grasshopper: A comparative studies of two methods

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### Abstract

The cytological studies of grasshoppers of Manipur was taken up for the first time and the creek grasshopper, *Gesonula punctifrons* (Stal) has 23 chromosomes in male germinal cells. The conventional squashed method was compared with the new modified technique of flame drying method. The meiotic cell division in grasshopper was distinct as reported and each cell stage could be easily identified in both the methods. The cells prepared with the squashed method were comparatively not uniformed and each chromosomes could not be spotted at a glance besides lesser stained while such difficulties could be overcome with the flame drying method. The minor demerits of flame drying method were: the limited numbers of cells and generally overstrained cells. The method is open to inspect whether this could be applied to other types of insects with somewhat smaller chromosome with addition of hypotonic solution to increase swimming areas of the chromosomes in future. During hypotonisation, the cells receive additional water due to osmosis, making them larger, the contents of the cell are loosened and chromosomes become more individualized.

**Keywords:** Manipur, *Gesonula punctifrons* cytology, squashed, hypotonic, testes, meiosis

### 1. Introduction

Grasshoppers are the most prevalent pests in all sorts of vegetation in pastures and grasslands. Family Acrididae encompasses the short-horned grasshoppers and locusts, phytophagous insects that are widely distributed throughout the world and considered ruinous in the arid zone [1]. But this species is cytologically very important because the male grasshoppers are using as a source for study meiotic division in some important university of India. The pioneer work of McClung [15] describing male grasshopper meiosis opened a long series of meiotic studies using grasshoppers as the preferred material. Despite seasonality of the male grasshoppers, *Gesonula punctifrons* (Stal) are ideal materials to study the various meiotic stages of spermatogenesis due to their easy availability, fairly large chromosomes, and fewer numbers of chromosomes [2]. The specimens are using to study both mitosis and meiosis in both under graduate and post graduate [3] mainly through the squashed method. It is easy to make temporary squash preparation of grasshopper testis with fixation and Acetocarmine stained. So this method is rapid, dependable and gives quick results. Some of drawbacks of this method is that the spread of chromosomes are not well uniformed and most of animal cells are not adequately stained and hamper clarity [3]. So the modified technique using acetic acid and centrifugation was reported by Banerjee [3] termed as flame drying method.

In present study comparative study of squashed method and the modified flame drying method of Banerjee [2] is reporting. The aim of the present study are to investigate the merits and demerits of the flame drying method over the squashed method and to test the effect of hypotonic solution on the clarity and individuality of the chromosomes. The method is open to inspect whether this could be applied to other types of insects with somewhat smaller chromosome with addition of hypotonic solution to increase swimming areas if the chromosomes in future.

### 2. Materials and Methods

The fifteen male grasshoppers of *Gesonula punctifrons* were collected from the Lamphelpat in the month of October, 2017 and brought to laboratory for further investigation. For the identification of the insects keys given by Shishodia [5] are used: Eyes large; antennae longer than head and pronotum together; pronotum long, narrow and rugose; supra-anal plate spoon-shaped, wide basally, with wide longitudinal median groove; apical part of hind tibiae modified

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## 2.1 Chromosome preparation

### 2.1.1 Squashed preparation

The testis were dissected out and put in fixative (Carnoy's fluid I) comprising of 1 part glacial acetic acid and 3 parts of ethanol by v/v for 24 hours. The seminiferous tubule were stained with 2% acetocarmine for 30 minute and softened with 45% glacial acetic acid. The tubules were covered with coverslide and finally applied thump pressure by folding in between the blotting paper [16].

### 2.2 Flame drying method

The chromosomes were prepared according to Banerjee [3] with modifications. The testis was dissected out in KCl (0.56M) and yellowish fat and other unwanted materials were taken out. This make the exposure the cells to KCl atleast for 20 minutes. Then the cells were treated to 45% Glacial acetic acid for 5 minutes and make slight crushing with micro pestle in the micro centrifuge tube and aspirated five times. The cell

## 2.3 Statistical analysis

Table 1

Parameters (mm)	Length of body	Length of antenna	Length of pronotum	Length of tegmen *	Length of hind femur	Length of hind tibia
Kolkata	18.2	9.4	3.9	17.8	11	9
Manipur	15.3	7.6	2.5	14.3	9	8

\*Tegmen a sclerotized forewing serving to cover the hindwing in grasshoppers and related insects

Only the male species were measured. In total 15 specimens were taken for the measurement. The criteria were depicted in the table (Table 1) and the measurements were compared with the data from Dey and Hazra [7].

## 3. Results and Discussion

The identity of the present study material *Gesonula punctifrons*, was in accordance with Dey and Hazra [7] and Srinivasan and Prabakar [8]. The species is reported also from Manipur earlier [4]. The species was a little smaller in comparison with the species found in Kolkata as depicted in Table (Fig. 1). This might be due to error measurements or whether in reality the species found in Manipur was smaller, was yet to be confirmed in future works.

As reported the 2n of the male grasshopper is 23 as reported [3, 9, 10]. The karyokinetic morphological changes occurring in seminiferous tubules of grasshopper testes were quite beautiful. The morphological changes that occur during the pairing of meiotic chromosomes were the basis for dividing prophase I into five sequential stages—Leptotene, Zygotene, Pachytene, Diplotene, and Diakinesis [6] and these are the characteristics of the division. The details of each stages could found elsewhere [5, 6]. The meiotic stages could be identified easily in both the methods: squashed and modified flame drying method (Fig. 2 and 3). The method was employed to study grasshopper chromosome by many researchers [3, 10-14]. Squashed method [Belling 1921] was one of the most rapid, easy and simple method that employed for the understanding the chromosomes of plant or animal materials in general [15]. The acetocarmine staining and squashed method was rather imperfect particularly for the grasshopper as reported by Banerjee [3].

The present study was quite similar to the Banerjee [3] but only different was the materials were dissected out in the hypotonic solution (KCl) which increases the more spaces for swimming area for individual chromosomes. The present studies showed much better chromosomes arrangements and

suspension was centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and pellet was carefully aspirated with a pipette so as to avoid clumping with fresh fixative and keep for 10 minute at room temperature. The centrifuge again at 5000 rpm for 10 minute and pellet was re-suspended with fresh fixative and spread on pre-chilled slides soaked with methanol. The slide was burn for 20 seconds then stored in a desiccator for three days [3].

### 2.2.1 Staining

The slides were stained in 2% Giemsa stained for 20 minutes and let dried. After staining the slides were dried in room temperature and made permanent with DPX with 22X60 cover slide and dried till the DPX was properly dry.

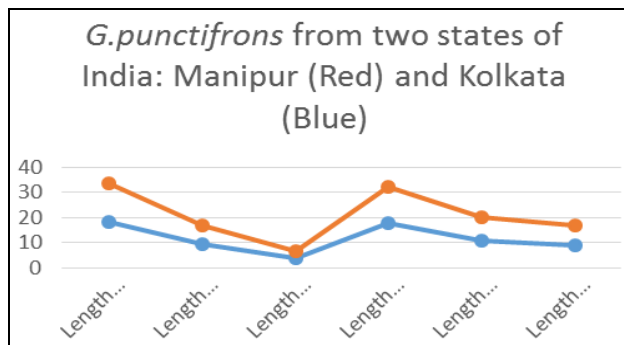
The slides were then mounted on microscope and inspects stages of meiosis. The selected stages were taken snaps at 40X with Coolpix digital camera attached to the microscope.

well separated stages. This might be the inclusion of the hypotonic solution. During hypotonisation, the cells receive additional water due to osmosis, making them larger, the contents of the cell are loosened and chromosomes become more individualized. Chromosomes can be damaged or washed away during final dissociation in case of excessive hypotonic treatment. However, chromosomes are still too compact and are not analysable in insufficiently hypotonised cells [17].

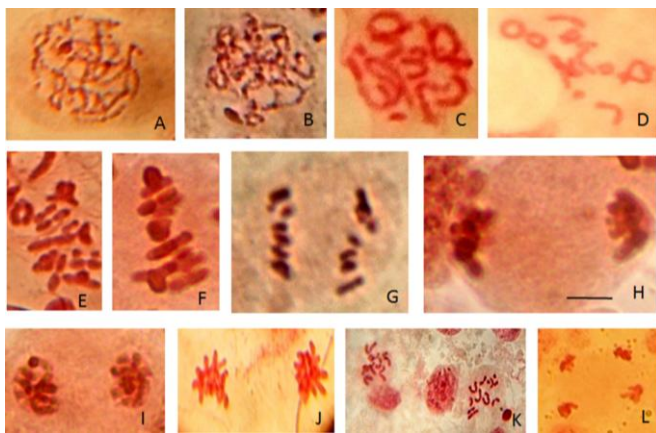
The spreading needs manual skill in suspension droplet movement on slide after dissociation. Unsuitable manipulation could lead to loss, damage or overlap of chromosomes. On the other hand, a squashed tissue could be easily insufficiently spread and then the chromosomes on slides could be poorly, or not at all analyzable, or even the tissue can be lost during coverslip removing. The use of squashing can be very problematic in organisms with high chromosome number [17].

The stages of the division were much higher in numbers in squashed method than the flame drying method. But the clarity and uniformity were much better in the flame drying method. But certain stages like Anaphase I and Telophases both I and II were hard to find in compare to squashed methods. The advantage of the flame drying method are: Clearer, uniformly flattened, efficiently stained, the chromosomes are up to the standard this might be the penetrability of the Giemsa stains than acetocarmine stain and finally the more prominent spermatogonial mitosis are quite magnificent (Fig. 3 K, L, M and N). The minor demerits of flame drying method are the cells are limited in number and need some practice to increase its applicability and generally overstrained. These stages were not quite beautiful and seem to be stained properly with Giemsa than acetocarmine. Some of the precautions to be bear in mind are: fixation should be slow and with minimum amount of the fixative for the first time in process, final dilution should be not more than 0.5 ml so as to make at least five slide and burning should be reduced

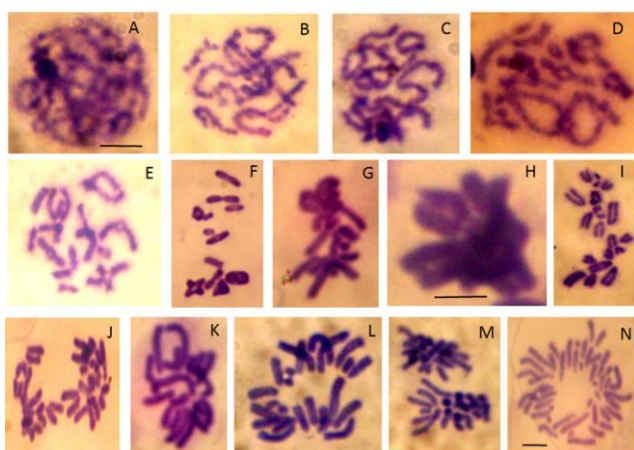
to prevent over spreading and staining should be for 5 to 10 minutes as to prevent overstraining. The method is open to insect whether this could be applied to other types of insects with somewhat smaller chromosome with addition of hypotonic solution to increase swimming areas if the chromosomes in future.



**Fig 1:** The comparative morphometric of the *G. punctifrons* from Kolkata and Manipur on the basis of Table 1.



**Fig 2:** The various stages of meiosis from seminiferous tubules of *Gesonula punctifrons* (Stal) obtaining from the acetocarmine staining. The meiosis comprising of Leptotene (A), Zygotene (B), Pachytene (C), Diplotene (D), Diakinesis (E), Metaphase I (F), Anaphase I (G) and Telophase I (H) of Meiosis I are vividly visible. The Meiosis II comprises of Prophase II (I), Metaphase II (J), Anaphase II (K) and Telophase II (L). Bar represents 5  $\mu$ m.



**Fig 3:** The various stages of meiosis from seminiferous tubules of *Gesonula punctifrons* (Stal) obtaining from the modified centrifuged method and Giemsa staining. The meiosis comprising of Leptotene (A), Zygotene (B), Pachytene (C), Diplotene (D), Diakinesis (E), Metaphase I (F), Prophase II (G) Metaphase II (H), early Anaphase II or late metaphase II (I) and Anaphase II (J) of Meiosis are more clearer and much enlarge besides isolation of each single cell. Spermatogonial mitosis are easily identifiable. It comprises of Prophase (K), Metaphase (L), Anaphase (M) and Telophase (N). Bar represents 5  $\mu$ m.

#### 4. Conclusions

The popular squashed method is not up to the mark for the study of grasshopper besides its simplicity, rapidity and quickness. The shortcomings of the squashed method could be easily replaced by flame drying method for its clarity, uniformity of the chromosomes and durability for the future used. The method is open to insect whether this could be applied to other types of insects with somewhat smaller chromosome with addition of hypotonic solution to increase swimming areas if the chromosomes in future. The method is open to inspect whether this could be applied to other types of insects with somewhat smaller chromosome with addition of hypotonic solution to increase swimming areas if the chromosomes in future.

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