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An ecological, morphological, and chromosomal study of *Dysdercus koenigii* (Pyrrhocoridae: Hemiptera) in Kurukshetra, Haryana

Paramjeet Kaur[1], Nidhi Kakkar[2]
[1] Department of Zoology, Kurukshetra University, Kurukshetra, Haryana, India
[2] Department of Zoology, IIHS, Kurukshetra University, Kurukshetra, Haryana, India

Abstract
The experimental species *Dysdercus koenigii* (Fabricius) belongs to family Pyrrhocoridae and order Hemiptera. It is also known as Red cotton bug and Cotton Stainer insect. In India it the main pest of cotton and Malvaceous Family. A total of 120 samples of *Dysdercus koenigii* adults and different stages of nymphs were collected randomly from fields of Kurukshetra University Campus, Kurukshetra, Haryana. The samples were identified with the help of identification keys. Life cycle and different ecological parameters were studied along with their morphometric and Cytological analysis. The cytogenetic data of *Dysdercus koenigii* was first time reported with diploid number of chromosomes 18 and sex chromosome mechanism "X_1X_2" in male individuals.

Keywords: *Dysdercus*, *koenigii*, Pyrrhocoridae, Hemiptera, Cotton stainer, Bug

Introduction

As we know Indian economy relies heavily on agriculture. 58% of population depends on agriculture for employment. The agriculture contributed in GDP was nearly 19.9% in 2020-21. India is the biggest exporter of cotton in the world. Its rank is second in the world in agriculture production (INSIGHTSIAS: insiteonindia.com).

Because of plain geography and healthy irrigation facilities, Punjab and Haryana states contribute their majority in food basket of India. These two states are agriculturally developed states of India (Singh and Singh, 2017) [36]. A minor pest of cotton in the North zone, the red cotton bug thrives in Haryana, where conducive conditions allow it to grow (Sandhu and Chhabra, 2001) [33]. The adult stage of the insect spends the winter while it remains active in all other developmental stages throughout the year.

Boll bursting stage of cotton plant get seriously affected by Red cotton bug which results in the production of poor quality of lint. The bugs stain the cotton lint with their body juices or excreta, which get crushed in the ginning factories. By staining the lint of cotton bolls; Red cotton bug also initiates the growth of certain bacteria inside the bolls (Sprenkel 2000 [37] and Arora *et al*. 2020) [2]. Seeds of developing cotton bolls are infested mainly by nymphs and adults, leaving a stain on lint. As a result, when the insect feeds by piercing flower buds or young cotton bolls, it typically leads to a decrease in size or may cause the fruiting body to abort and fall to the ground. Hollyhock serves as an alternative host for Dysdercus koenigii. (Kamble 1971 [15] and Sprenkel 2000) [37]

So far, only 22 species in 7 genera of Pyrrhocoridae have been studied from a cytogenetic perspective (Papeschi and Bressa 2006 [26], Verma and Kurl 2009 [40], Bardella *et al*. 2014). In addition to the general characteristics of heteropterans, such as holokinetic chromosomes and post-reductional meiotic division of sex chromosomes, this family has a modal diploid chromosome number of 16 (♂), with a range of 12 to 33 autosomes and simple (X0), multiple (X_1X_20), as well as neo-sex chromosome systems (neoX-neoY). Out of 22 species in the Pyrrhocoridae, 11 have the ancestral X0 sex chromosome system (Papeschi and Bressa 2006 [26], Verma and Kurl 2009) [4]. Eight species of *Dysdercus* have been reported to have multiple sex chromosomes (X_1X_20) (Piza 1947 [28], Suman 2010) [38], a species of *Pyrrhopeplus* (Parshad 1957) [27] and a species of *Odontopus* (Verma and Kurl 2019) [4], whereas a neo-X neo-Y sex chromosome system was described in another species of *Dysdercus* (Bressa *et al*. 1999 [7], Bressa *et al*. 2009) [8]. In terms of the behavior of the sex chromosomes, the Pyrrhocoridae family has been studied extensively.

A sex-determining mechanism based on X_1X_20 shows very peculiar behavior when the two Xs are present together. In the diffuse stage, they remain fused, separate at diplotene, lie apart during metaphase I, divide equationally during anaphase I, rejoin during metaphase II, and move to one of the poles together during anaphase II. Based on this phenomenon, two sex bodies as X and Y have been identified and confirmed meiosis as double reductional (Ray-Chaudhuri and Manna 1952) [31]. The concept of post-reductional meiosis, which has since become widely accepted, was proposed in contrast to this situation (Battaglia 1956) [6]. Different species exhibit different degrees of association between X1 and X2. In *D. cingulatus* (Fabricius) (Sharma 1956) [35] and *D. mendesi* (Piza 1947) [28]. Two Xs merely approach each other, whereas in *D. koenigii* (Fabricius), they unite to create a singular, constricted element (Battaglia 1956 [6], Sharma 1956) [35], and fuse to form a single mass in *P. posthumus* Horváth (Parshad 1957) [27]. The diploid number of *O. nigricornis* Stal has been reported as 2n = 12 = 10A + X_1X_20, but this claim is not clear from the supporting photographic evidence (Verma and Kurl 2009) [40].

2. Material and Method

Adults and nymphs of *Dysdercus koenigii* were gathered indiscriminately from the grassy fields of Kurukshetra University campus, Kurukshetra. They were collected in 70% alcohol and brought to the laboratory for further analysis. The samples were identified with the help of identification keys (Kapur and Vazirani 1956) [16]. Life cycle and different ecological parameters were studied along with

their morphometric and Cytological analysis.

Male specimens of Dysdercus koenigii were employed for cytological preparations. Adult male bugs were euthanized in a 0.56% KCl solution. The testicular material, upon extraction, was exposed to a 0.001% colchicine solution for 20 minutes, followed by immersion in a 1% sodium citrate solution for another 20 minutes at room temperature. Subsequently, the material underwent hypotonic treatment and was then fixed in a cold 1:3 acetic-methanol solution for 20 minutes, with 2-3 changes. The fixed material was utilized for slide preparation using the air-drying method for insects (Yadav and Lyapunova 1983) [42].

After adequate screening of slides under Olympus CX41 microscope, photomicrographs of selected stages were taken using oil immersion objective (100X) and digital compact camera (Olympus, C-7070). From the printer generated selected photomicrographs; different stages of meiosis and sperm developmental stages were studied.

3. Results and Discussion

A total of 120 samples were collected from the University Campus, Kurukshetra University, Kurukshetra. The samples were identified as *Dysdercus koenigii*. It belongs to Family Pyrrhocoridae and order Hemiptera. It is a polyphagous pest of cotton, millets, Malvaceous and Bombaceae families (Kamble 1971 [15], Fakri and Sader Alam 2005). It is distributed in various parts of world: India, Pakistan, Sri Lanka, Burma and South East Asia (Freeman 1947 [12], Kamble 1971 [15], Wadnerkar *et al*. 1979 [41], Ahmed and Mohammad 1983 [1], Jallel *et al*. 2013) [14]. The insects are elongated and slender, characterized by a crimson-red coloration with white bands running across the abdomen. The bugs were abundant during the hot days. Mating individuals were also observed in these days. The individuals were less in number in cloudy and rainy days. The membranous portion/ tip of their fore wings, antennae are black. Antennas are 4 segmented as shown in Fig 1.

Fig 1: Antenna of D.Koenig ii

Adults were observed with end to end position mating during the day time. The mating may occur with immature females also (Fig 2). The colour of eggs was creamy-white which turn to yellowish orange before hatching. During the field observation 5 nymphal instars were observed in *D. koenigii* (Fig 3). Life cycle of *D. koenigii* has been studied by observing different developing stages (Fig 5).

Fig 2: D.koenigii (ma ti ng)

Fig 3: D.ko enigii (Nymphal instars)

First Nymphal Instar is light orange in colour. Its length is 2mm and width is 1mm. wing pads were absent in first instar. First nymphal instar may change its colour from orange buff to red [33].

Second Nymphal instar is larger than first instar. It is orange in colour. Its length is 3mm and width is 1mm and without wing pads.

Third Nymphal instar is developed than first and second instar in having emergence of wing pads. It is orange in colour. Its length is 6mm and width is 2mm.

Fourth Nymphal instar is crimson red in colour. Wing pads developed upto posterior margin of meta thorax. Posterior tip of wing pad darker in colour than proximal parts. Its length is 8mm and width is 4mm.

Fifth Nymphal instar is with prominently developing darker wings. It is crimson red in colour. Its length is 10mm and width is 3-4mm.

Adults formed after the formation of 5 nymphal instars. Adults of *Dysdercus koenigii* are crimson red in colour. Hind wings are membranous and broader than fore wings. Hind wings remained concealed under fore wings at rest.

Fig 4: D.koenigii Female genitalia

Male and female bugs show sexual dimorphism. The male is smaller than female and tip of abdomen is pointed in male and slightly blunt in female bug (Fig 4). In female genitalia is not well developed.

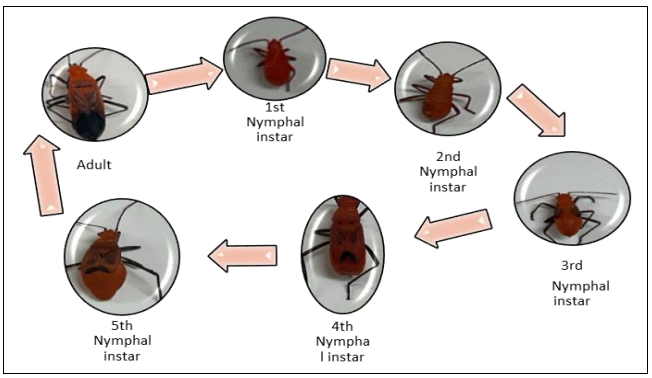


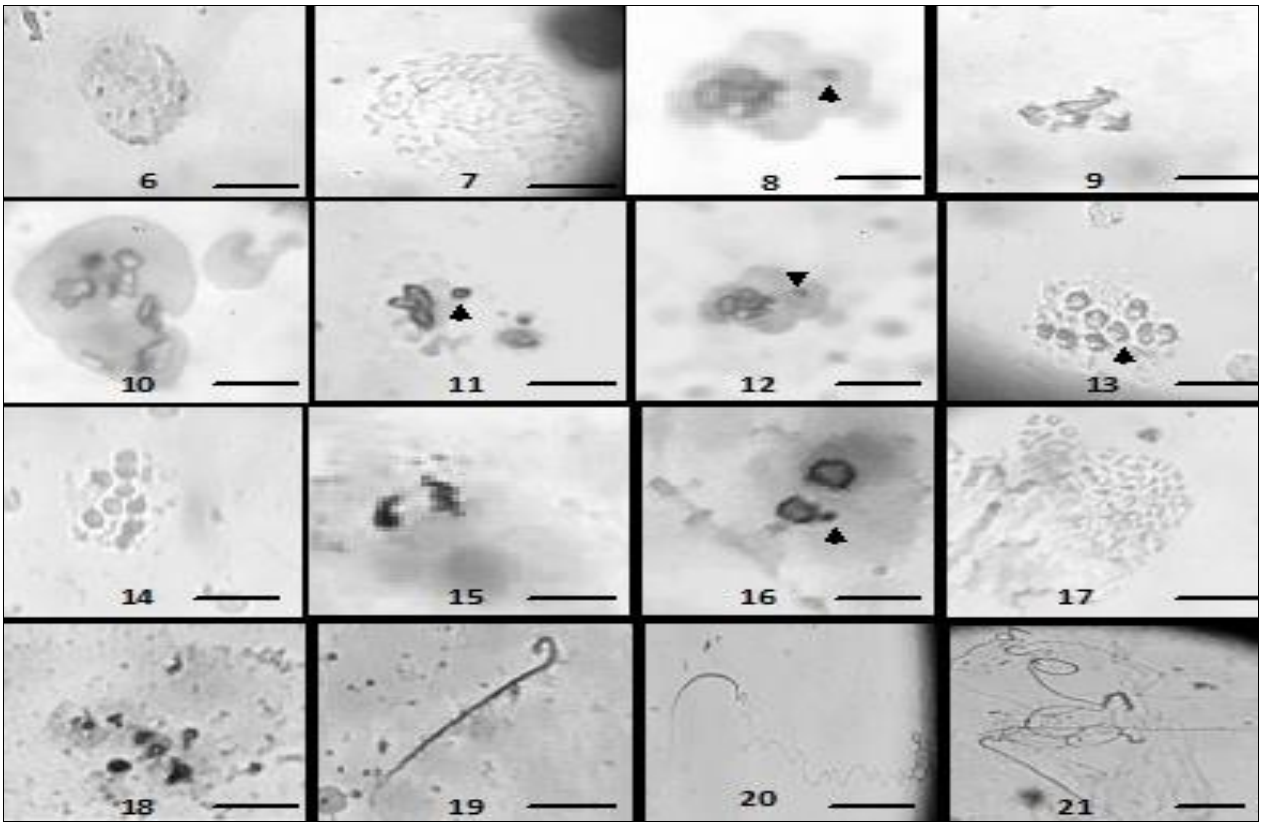
Fig 5: Life cycle of *Dysdercus koenigii* with 5 Nymphal instar and Adult

Dysdercus koenigii is found to have a male diploid chromosome complement of 2n = 18 = 16A + X_1X_2. The sex chromosomal complement was represented by a positively heteropycnotic body during the early stages of meiosis I (Fig 8-11). Multiple sex chromosome system had also been confirmed in this family (Kaur and Gaba 2015). However, the autosomal elements vary and overlap in their sizes. The smallest elements represent two sex chromosomes. *Dysdercus koenigii* shows cytologically a heteromorphic pair of sex chromosomes. This difference in the size of two sex chromosomes in had also been repoted earlier in *Dysdercus cingulatus* (Sharma 1956) [35].

The typical course of meiosis in the male *Dysdercus koenigii* follows the pattern observed in heteropteran insects. The darkly stained heteropycnotic mass is observed during early prophase I and in diffused stage of Prophase II (Fig 17), which represents the sex chromosomes. Diplotene stage shows the ring shaped and rod shaped elements which depicts the formation and terminalisation of chiasmata (Fig 10). During diakinesis each sex chromosome get split out and along with the autosomes occupy the periphery of the nucleus (Fig s 11 & 12). At metaphase I, the autosomal bivalents are observed to be arranged in the form of dumbbell shaped elements (Fig s 13 & 14). All the bivalents undergo co-orientation and the sex bivalent chromosomes show auto-orientation. The anaphase I is again observed to be very typical with movement of the elements towards each pole of the spindle (Fig 15). Sex chromosomes are lying very close to each other and get fused to form a single element in the centre of each anaphasic group of autosomes. Telophase I revealed very clear demarcations in autosomes and fused sex chromosomal body (Fig 16).

Somatic number of chromosomes has been observed as 18 including X_1 and X_2. The sex chromosomes during the meiotic division segregate to the opposite poles of the spindle in the first meiotic division. The second meiotic division reveals either X_1 or X_2, which forms an accessory plate and passes undivided to one pole (Fig 18). This results in the formation of two types of sperms one with X_1 chromosome and the other with X_2 chromosome.

The result presented in this paper provide a strong evidence in support of earlier reports (Battaglia 1956 [6] and Sharma 1956) [35] for the interpretation of the meiotic number and sex chromosomes in *Dysdercus koenigii*. The two Xs fuse to form a single constricted element as shown in plate (Fig s 8, 10-12 & 16).



Dysdercus koenigii **Fig 6:** Laptotene; **Fig 7:** Zygotene; **Fig 8&9:** Pachytena; **Fig 10:** Diplotene; **Fig 11&12:** Diakinesis; **Fig 13&14:** Metaphase I; **Fig 15:** Anaphase I; **Fig 16:** Telophase I; **Fig 17:** Prophase II; **Fig 18:** Metaphase III; **Fig 19:** Spermatid; **Fig 20:** Sperm; **Fig 21:** Sperms wich hooked head (Bar=0.01 Um)

However, in other Pyrrhocoridae species such as *D. fasciatus* Signoret, *D. intermedius* Distant, *D. superstitiosus* (Fabricius), and *Pyrrhopeplus posthumus* Horváth, which possess multiple X chromosomes, all X chromosomes are situated within the autosomal ring during metaphase I but outside of it during metaphase II (Ray-Chaudhuri and Manna 1952 [31], Parshad 1957 [27], Sharma *et al*. 1957 [24], Banerjee 1958 [3], Ray-Chaudhari and Banerjee 1959 [30], Suman 2010) [38].

It has been noted in heteropteran species with an Xn0 sex chromosome system that multiple X chromosomes often merge into a single element throughout or for most of the meiotic process (Manna 1951 [22], Dutt 1957, Parshad 1957 [27], Fossey and Liebenberg 1995, Grozeva 2006, Suman 2010 [38], Kaur and Bansal 2012a, 2012b, Bansal and Kaur 2013). However, in cases like *Dysdercus*, which also possess an X_n0 multiple sex chromosome system, the number of X chromosomes can be determined in the spermatogonial complement because each chromosome exists as a separate entity (Manna 1957 [23], Manna 1984 [24], Kuznetsova 1988) [20].

A number of different developmental stages of sperms have also been encountered during the observations (Fig s 19-21). Sperms are bearing hooked shaped head and a long tail. No recent studies have been made on the cytogenetic analysis of these cotton strainers. As a consequence, there is a very limited amount of literature available. There is need to explore more and more cytogenetic analysis of these economically important cotton strainers, to establish their phylogenetic relationships with other species, which in turn will help to control these insects below the economic injury level by using integrated pest management.

Conclusion

Hemipteran, red cotton stainer is a serious pest of cotton in North zone and Haryana, India. It is active throughout the year. After five moults, it is metamorphosed into an adult. Adults show sexual dimorphism. Life cycle, temporal and spatial variations along with cytogenetic data of this economically important pest of cotton crop, *Dysdercus koenigii*, will be helpful for the integrated pest management strategies adopted by different farmers to control this pest below economic injury.

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