



แคร์ริโอไทป์ของตั๊กแตนสามชนิด
(Othoptera: Acrididae/Pyrgomorphidae) ในจังหวัดอุดรธานี
Karyotypes of three grasshopper species
(Othoptera: Acrididae/Pyrgomorphidae) in Udon Thani province

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บทคัดย่อ

การวิจัยมีวัตถุประสงค์เพื่อตรวจสอบแคร์ริโอไทป์ของตั๊กแตนบางชนิดในวงศ์อะคริดิตี (Acrididae) และในวงศ์ไพร์โกมอร์ฟิตี (Pyrgomorphidae) เก็บตัวอย่างตั๊กแตนเพศผู้ตัวเต็มวัยชนิดละ 10 ตัว ในพื้นที่มหาวิทยาลัยราชภัฏอุดรธานี ศูนย์การศึกษาสามพร้าว เตรียมโครโมโซมระยะเมทาเฟสจากเซลล์ที่กำลังมีการแบ่งตัวในอันทะ (สเปิร์มมาโทโกเนีย) ย้อมสีโครโมโซมแบบธรรมดาด้วยสีกิมซาส์ 10 เปอร์เซ็นต์ ผลวิจัยแสดงให้เห็นว่าตั๊กแตนหนวดสั้นปลายขาว (*Phlaeoba antennata*) มีจำนวนโครโมโซมดิพลอยด์ $2n = 23$ แคร์ริโอไทป์ประกอบด้วยโครโมโซมชนิดเทโลเซ็นทริกทั้งหมด มีสูตรแคร์ริโอไทป์ คือ $2n (23) = L^t_8 + M^t_2 + S^t_{12} + XO$ ตั๊กแตนหนวดสั้นสีตาลจาง (*P. infumata*) มีจำนวนโครโมโซมดิพลอยด์ $2n = 23$ แคร์ริโอไทป์ประกอบด้วยโครโมโซมชนิดเทโลเซ็นทริกทั้งหมด มีสูตรแคร์ริโอไทป์ คือ $L^t_{10} + M^t_8 + S^t_4 + XO$ ส่วนตั๊กแตนหน้าเอียงปีกส้ม (*Tagasta marginella*) มีจำนวนโครโมโซมดิพลอยด์ $2n = 19$ แคร์ริโอไทป์ประกอบด้วยโครโมโซมชนิดเทโลเซ็นทริกทั้งหมด มีสูตรแคร์ริโอไทป์ดังนี้ $2n (19) = L^t_6 + M^t_{10} + S^t_2 + XO$ ตั๊กแตนสามชนิดดังกล่าวมีการกำหนดเพศโดยระบบโครโมโซม XX/XO ข้อมูลเซลล์พันธุศาสตร์นี้สามารถใช้สนับสนุนทางอนุกรมวิธาน ซึ่งทำให้เกิดองค์ความรู้ที่เพิ่มขึ้นในเชิงความสัมพันธ์ทางวิวัฒนาการของแมลงกลุ่มนี้ในอนาคตต่อไป

ABSTRACT

This research aimed to examine karyotypes of some grasshopper species in the family Acrididae and Pyrgomorphidae. Ten adult male specimens of each species were collected in the area of Samprao campus, Udon Thani Rajabhat University. The metaphase chromosomes were prepared from mitotic dividing cells in testes (spermatogonia). Conventional staining was applied using 10% Giemsa's solution. The results showed that white-tipped grasshopper (*Phlaeoba antennata*) had a diploid chromosome number of $2n = 23$. Karyotype was consisted of all telocentric chromosomes, and karyotype formula was $2n (23) = L_8^t + M_2^t + S_{12}^t + XO$. Testaceous oblique-faced grasshopper (*P. infumata*) had a diploid chromosome number of $2n = 23$, karyotype comprised all telocentric chromosomes, and karyotype formula was $2n (23) = L_{10}^t + M_8^t + S_4^t + XO$. Orange-winged slant-faced grasshopper (*Tagasta marginella*) had a diploid chromosome number of $2n = 19$, karyotype was composed of all telocentric chromosomes, and karyotype formula was $2n (19) = L_6^t + M_{10}^t + S_2^t + XO$. Sex determination of these grasshopper species was XX and XO system. These cytogenetic data can be employed as a source of taxonomic information which is provide more details for evolutionary relationship knowledge of these insect groups in the future.

คำสำคัญ: แคริโอไทป์ ตั๊กแตน อะคริดิดี ไพรโกมอร์ฟิดี

Keywords: Karyotype, Grasshopper, Acrididae, Pyrgomorphidae

INTRODUCTION

Grasshoppers are a member of insects which are classified in the order Orthoptera. They are diverse as in other insect groups with approximately 11,000 species worldwide in 22 families, but the highest diversity is in the tropical region (Grzimek et al., 2004). At least 47 species of grasshoppers in the family Acrididae were found in Thailand (Chaweewan et al., 2007). White-tipped grasshoppers (*Phlaeoba antennata*, Brunner von Wattenwyl, 1893) are commonly found in short grassland. Its head is short and slanted-face, first pronotum keel is straight. Body color can be varied in brown and blue-green, and are remarkably with white on antennae tip (Figure 1A). Testaceous oblique-faced grasshoppers (*Phlaeoba infumata* Brunner von Wattenwyl, 1893) are widely spread in Thailand. Its body color is light brown, dark brown striped band run from its head, eyes, and end of its wing (Figure 1B). Both of *P. antennata* and *P. infumata* are common species of India and are also

distributed in other country for such as Bangladesh, East Nepal, Hainan Islands, Myanmar, South and North Malacca, South China, Tenasserim and Yunnan. They are a minor pest of paddy, sorghum, maize and millets. Both adults and nymphs are generally found throughout the year in moist area. Maximum population observed in the month of November. Probably it is three generation per year (Srinivasan and Prabakar, 2013). Orange-winged slant-faced grasshoppers (*Tagasta marginella*, Thunberg, 1815) inhabit in grassland with small shrub. The ecology, economy and other information of this species has been least study. This species can be found around the Himalayan mountains, southern China, Indo-China, Malesia, Philippines including in Thailand. Its head is quite tapering small, antennae base are flat and located between eyes, and on both head side have white-rough spots. Its pronotum have white edge, anterior wings are shorter than abdomen while posterior wings are orange (Figure 1C) (Ek-Amnuay,

2003). Grasshoppers in the family Acrididae and Pyrgomorphidae have been cytogenetically studied so far. They were reported mainly in East Asia such as China and Japan (Ma and Zheng, 1989; Ma et al., 1994; Yochimura et al., 2005), and South Asia such as India (Phimphan et al., 2017) whereas some species were reported in South-east Asia including Thailand (Phimphan et al., 2017; Phimphan and Sangpakdee, 2018). Knowing of genetic information at the chromosomal level are interested due to the data can be used to create the standard karyotype and genes mapping in the lather.

Even though these grasshoppers were not concerned in the red list of IUCN and some were

already reported, the classical cytogenetic data obtained herein will provide fundamental knowledge for the grasshopper group. Nowadays, the insecticides have still being used in agricultural farming; consequently there is a risk that orthopteran species of Thailand may disappear before their existence can be documented. In the present study, we therefore attempt to present the first karyotype report of two acridids and one pyrgomorphid species that inhabit in the area of Samprao campus, Udon Thani Rajabhat University, Thailand as database in order to use this information in the future study.

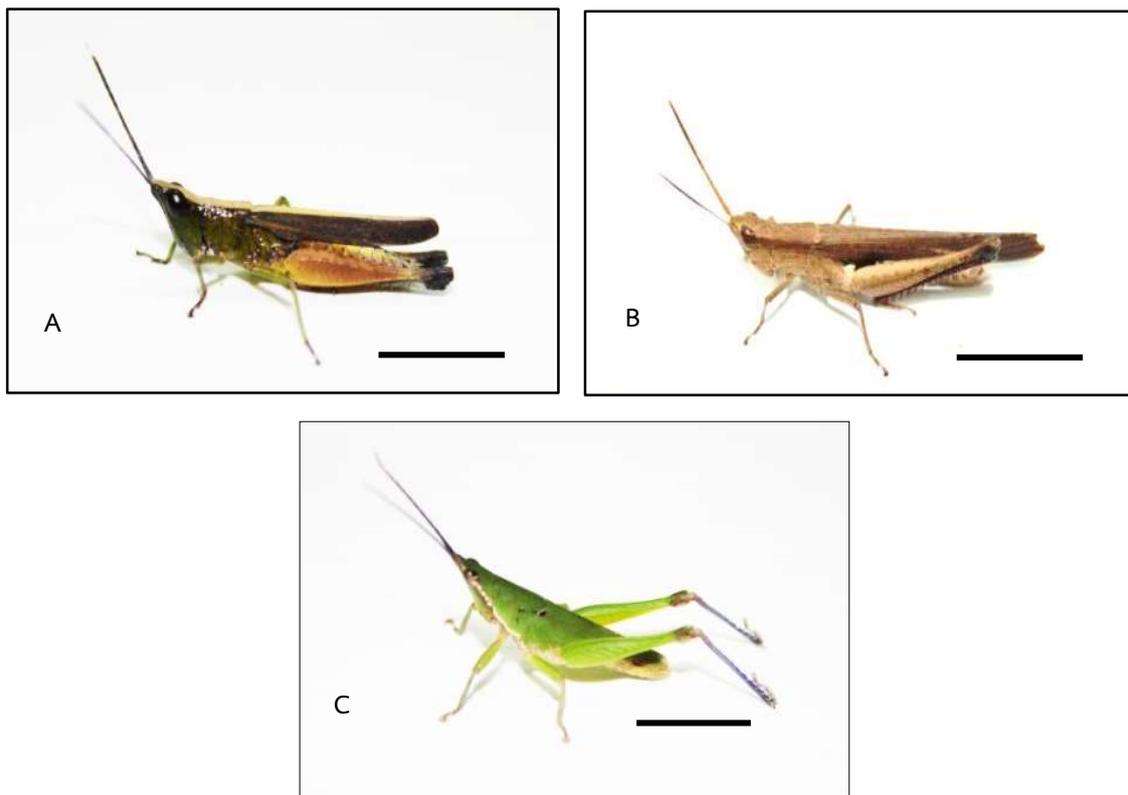


Figure 1. Common characteristic of adult male *Phlaeoba antennata* (A), *P. infumata* (B) and *Tagasta marginella* (C). Scale bars indicate 1 cm.

RESEARCH METHODOLOGY

Ten adult male specimens of three species were collected from June to September in 2020 in the area of Samprao campus, Udon Thani Rajabhat University (48Q 280915.39E, 1930596.83N), Udon Thani province, Thailand. The individual specimens were identified following that of Ek-Amnuay (2013) Mitotic chromosomes were prepared from testes by means of colchicine hypotonic cell suspension air drying technique (Phimphan et al., 2017). Briefly, the testes were dissected out after injection of 0.01% colchicine for an hour, and were then gently minced in 0.075 M KCl. The cell suspensions were incubated for 30 minutes and were then centrifuged at 3,000 rpm for 10 minutes, and the supernatant was discarded. After that, the cell suspensions were fixed with fresh-cold fixative (methanol and acetic acid in 3:1) and the supernatant was discarded after centrifugation for three times. The cell suspensions were spread on a clean slide and air dried. Conventional staining was applied using 10% Giemsa solution for 15 min. The high qualities of ten metaphases from all ten individual specimens of each species were investigated and captured under the Olympus CX22LED with CCD camera. Cytological analyses were computed according to Chaiyasut (1989).

RESULTS

Phlaeoba antennata, *P. infumata* and *Tagasta marginella* had diploid chromosome numbers

of $2n = 23, 23$ and 19 , respectively. The two *Phlaeoba* species (family Acrididae) had the same fundamental numbers (NF) of 23 , while *T. marginella* (family Pyrgomorphidae) had $NF = 19$. An XX/XO sex-determining mechanism was presented in all of them. The chromosomes could be divided into three size groups according to their relative lengths. The karyotypes of all three species were composed of all telocentric chromosomes. Karyotype of *P. antennata* comprised 4 pairs of large chromosomes (L1-4), 2 pairs of medium chromosomes (M5), 6 pairs of small chromosomes (S6-11) and one medium X chromosome. The karyotype formula was $2n (23) = Lt8 + Mt2 + St12 + X$ (Figure 2A and 2B).

Karyotype of *P. infumata* was composed of 5 pairs of large (L1-5), 4 pairs of medium chromosomes (6-9), 2 pairs of small chromosomes (10-11) and one large X chromosome. The karyotype formula was $2n (23) = Lt10 + Mt8 + St4 + X$ (Figure 2C and 2D). Karyotype of *T. marginella* was consisted of 3 pairs of large chromosomes (L1-3), 5 pairs of medium chromosomes (4-8), 1 pair of small chromosomes (9) and one X chromosome that was similar in size to large autosomes. The karyotype formula was $2n (19) = Lt6 + Mt10 + St2 + X$ (Figure 2E and 2F). The average of chromosomal sizes and their relative lengths of three species are presented in Table 1, Table 2 and Table 3, respectively.

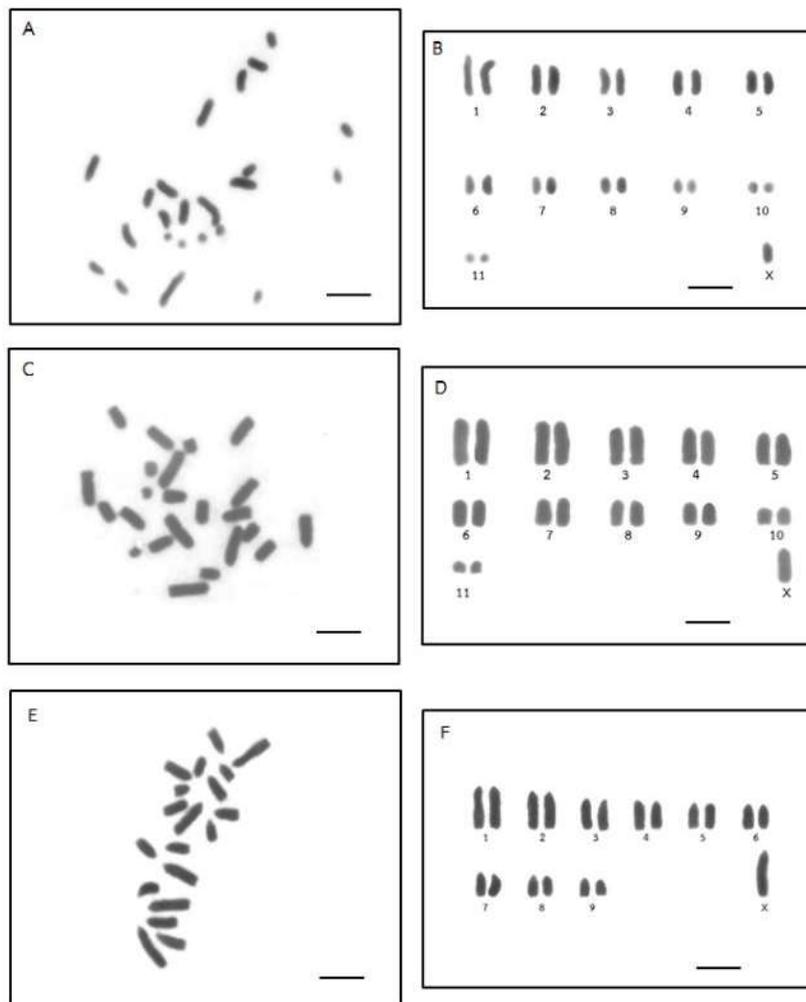


Figure 2. Mitotic metaphase chromosome plates and karyotypes of *Phlaeoba antennata* (A and B) $2n = 23$, *P. infumata* (C and D) $2n = 23$ and *Tagasta marginella* (E and F) $2n = 19$ by conventional staining. Scale bars indicate 20 μm .

Table 1 Chromosome length of LT, relative lengths (RL), and chromosome size groups in *Phlaeoba antennata*

Parameter	Telocentric chromosome pairs											
	1	2	3	4	5	6	7	8	9	10	11	X
Mean of chromosome length of LT (μm)	19.60 ± 0.12	16.57 ± 0.15	13.79 ± 0.20	12.32 ± 0.15	10.86 ± 0.13	9.14 ± 0.18	8.27 ± 0.13	7.76 ± 0.16	6.20 ± 0.10	5.40 ± 0.10	3.97 ± 0.12	10.56 ± 0.16
Mean of RL (LT/ Σ LT)	0.157 ± 0.013	0.133 ± 0.008	0.110 ± 0.006	0.099 ± 0.006	0.083 ± 0.010	0.073 ± 0.004	0.066 ± 0.004	0.062 ± 0.003	0.050 ± 0.005	0.043 ± 0.002	0.032 ± 0.003	0.086 ± 0.010
Chromosome size groups		L			M			S				M
Mean lengths (μm)		15.57			10.86			6.79				10.56
* Chromosome size lengths	L 11.78 \leftarrow M \rightarrow 9.80 S											

Table 2 Chromosome length of LT, relative lengths (RL), and chromosome size groups in *Phlaeoba infumata*

Parameter	Telocentric chromosome pairs											
	1	2	3	4	5	6	7	8	9	10	11	X
Mean of chromosome length of LT (μm)	23.56 ± 0.23	21.33 ± 0.20	19.78 ± 0.18	17.81 ± 0.12	15.78 ± 0.16	14.10 ± 0.18	14.01 ± 0.20	13.46 ± 0.16	11.98 ± 0.14	9.05 ± 0.10	6.07 ± 0.10	17.53 ± 0.22
Mean of RL (LT/ Σ LT)	0.128 ± 0.001	0.116 ± 0.003	0.107 ± 0.001	0.097 ± 0.002	0.086 ± 0.002	0.076 ± 0.001	0.076 ± 0.001	0.073 ± 0.002	0.065 ± 0.002	0.049 ± 0.003	0.033 ± 0.003	0.095 ± 0.001
Chromosome size groups	L			M				S		L		
Mean lengths (μm)	19.65			13.39				7.56		17.53		
* Chromosome size lengths	L 14.81			← M				→ 11.78		S		

* Large (L) chromosome = the chromosome length of $\geq (LT^1+LT^{11})/2$

Medium (M) chromosome = the chromosome length between $\leq (LT^1+LT^{11})/2$ and $\geq LT^1/2$

Small (S) chromosome = the chromosome length of $\leq LT^1/2$

Table 3 Chromosome length of LT, relative length (RL), and chromosome size groups in *Tagasta marginella*

Parameter	Telocentric chromosome pairs										
	1	2	3	4	5	6	7	8	9	X	
Mean of chromosome length of LT (μm)	17.46 ± 0.22	15.39 ± 0.18	13.33 ± 0.20	12.27 ± 0.24	11.48 ± 0.12	10.87 ± 0.16	10.37 ± 0.15	9.37 ± 0.12	7.94 ± 0.10	18.98 ± 0.14	
Mean of RL (LT/ Σ LT)	0.152 ± 0.032	0.132 ± 0.022	0.112 ± 0.019	0.103 ± 0.017	0.097 ± 0.018	0.091 ± 0.014	0.087 ± 0.014	0.078 ± 0.014	0.068 ± 0.013	0.161 ± 0.023	
Chromosome size groups	L			M				S		L	
Mean lengths (μm)	15.39			10.87				7.94		18.98	
*Chromosome size lengths	L 12.70			← M				→ 8.73		S	

* Large (L) chromosome = the chromosome length of $\geq (LT^1+LT^9)/2$

Medium (M) chromosome = the chromosome length between $\leq (LT^1+LT^9)/2$ and $\geq LT^1/2$

Small (S) chromosome = the chromosome length of $\leq LT^1/2$

CONCLUSION AND DISCUSSION

The two species of *Phlaeoba* analyzed in this present report had similar diploid chromosome numbers ($2n = 23$), chromosome characteristics and sex determining mechanism. These were also found in many acridids, e.g. in the genera *Abracris*, *Eujivarus*, *Eusitalces*, *Psilocirtus*, *Xiphiola* (Ferreira et al., 1980; Mesa et al., 1982; Cella and Ferreira, 1991), *Roppacris* (Mesa and Fontanetti, 1983), *Oxya* (Ma and Zheng, 1989; Ma et al., 1994; Yoshimura et al., 2005;

Phimphan et al., 2017), *Orthoscapheus* (Rocha et al., 2011), *Caryanda* (Quing et al., 2012), *Acrida*, *Cyrtacanthacris*, *Gastrimargus*, *Hieroglyphus*, *Parahieroglyphus* and *Pseudoxya* (Koli et al., 2013; Phimphan and Sangpakdee, 2018; Phimphan et al., 2018). This suggests that the diploid chromosome number of $2n = 23$ for short-horned grasshoppers tend to be the model karyotype of this family. Therefore the two species cytogenetically studied herein followed that of the model Acrididae karyotype which

showed cytogenetic uniformity regarding diploid number and XX/XO sex determining mechanism. However, some acididid species have shown the diploid number of $2n = 19$ chromosomes in *Jodacris f. ferrugineus*, *J. chapadensis*, *J. furcillata* and *Sitalces volxemi* (Ferreira et al., 1980; Mesa et al., 1982).

A great cytogenetic uniformity of short-horned grasshoppers has been considered as an example of "Karyotypic conservatism" (Aswathanarayana and Ashwath, 2006). Although many species in acrididids had been reported so far regarding its karyotypes were consisted of all acrocentric/telocentric chromosomes, each species is still unique and provides an karyotypic identity to species (Channaveerappa and Ranganath, 1997). In the present study, the karyotype of two *Phlaeoba* species showed some differences in karyotype formulae, due to the chromosome sizes that can be divided into three size groups according to their chromosome size lengths: large, medium and short. Moreover, both species also showed different sizes of sex-chromosome with a medium X chromosome for *P. antennata* and a large X chromosome for *P. infumata*. The chromosomal characteristics and morphology studied herein were also differed from the previous studied (Chadha and Mehta, 2011) that reported the karyotypes of these two *Phlaeoba* sp. were composed of all acrocentric chromosomes. The chromosome size lengths were described as 3 pairs of large chromosomes (L1-3), 6 pairs of medium chromosomes (M4-9) and 2 pairs of small chromosomes (S10-11), and one largest X chromosome in both species. We suggests that these differences may be caused by population polymorphism.

In this article, the chromosome complements of *T. marginella* was described for the first time in Thailand. This species had a diploid number of $2n =$

19, all chromosomes were telocentric. The diploid number was presented to be similar to that of some pyrgomorphid species, e.g. *Atractomorpha crenulata*, *Chrotogonus trachypterus* and *Poecilocerus pictus* (Sandhu and Chadha, 2012). Moreover, The members of Pyrgomorphidae have been revealed to have a highly conserved of chromosomes complement made up of 19 acrocentric chromosomes in male individuals (White, 1973; Hewitt, 1979). In addition, acrocentrism has been shown to be quite primitive feature in evolutionary stock which gives rise to Acridoidea (White, 1973). The evolution of karyotype has been attributed to Robertsonian fusion and fissions, inversions, and translocations. In other species where chromosome number has been reduced below $2n = 23$ without the metacentric elements, this presumably that this phenomena can be occurred by fusions then followed by pericentric inversions. This also seems to be probably the way that lead the pyrgomorphid karyotype $2n = 19$ was originated from $2n = 23$ karyotype (Sandhu and Chadha, 2012). Although the female samples of three species were not reported herein, The sex-determining mechanism is presumed to be XO/XX type among all the studied species. Such as, the sex-mechanism found in Haryana population Acridoideans (Yadav and Yadav, 1986) and some in 11 species of grasshoppers from Simla (H.P), Sharma and Gautam (2002) also revealed similar results. Therefore, the short horned grasshoppers of different regions are showing cytogenetic uniformity regarding sex-determining mechanism.

This study we suggest that studies concerning the tandem sequences repeats such as telomeric TTAGGG (n), 18S, 5.8S and 25S rRNA sequences are needed to help understanding chromosomal evolution changes in the orthopterans in the future study.

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