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ORIGINAL ARTICLE

Chromosomal analysis and meiosis studies of *Oxya chinensis* (Orthoptera: Acrididae) from Thailand

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Abstract The standard karyotype and meiotic cell divisions of *Oxya chinensis* Thunberg, 1815 (Orthotera: Acrididae) from Khon Kaen, Thailand were studied by conventional and silver staining techniques. Chromosomal preparations were made from grasshopper testes. The results showed that the male *O. chinensis* had diploid chromosome number $2n = 23$ (XO). Karyotype was presented with eight long (1–4 pairs), eight medium (5–8 pairs) and six short (9–11 pairs) acrocentric chromosomes with minute short arms in all autosomes. The acrocentric X chromosome was approximately same in size as found for medium autosomes. The nucleolar organizer regions (NORs) were found on telomeric regions of all minute short arm acrocentric chromosomes in metaphase, including X chromosome. In zygotene stage of meiotic cell division, NORs were detected in three regions per cell by silver staining while only one region was found by conventional staining. Meiotic cell divisions were also studied to confirm the diploid chromosome number and normal meiotic behaviour. The karyotype formula was found in *O. chinensis*: $2n = 23$ (XO); $La\ 4 + Ma\ 4 + Sa\ 3 + X$-chromosome.

Keywords Grasshopper · Karyotype · *Oxya chinensis*

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Introduction

The small Chinese rice grasshopper (*Oxya chinensis* Thunberg, 1815) (Fig. 1) is a major insect pest in rice, occurs in all rice—growing areas of Thailand. The male adults are brown, greenish yellow grasshoppers, with about 25–30 mm body length. Females are slightly larger, with a conspicuous broad brown stripe laterally through the eyes and extending posteriorly along tegmina. The prominent distal end of hind tibia is dark brown. Males generally have six instars whereas females have 6–7 instars. Eggs are generally laid in a pod just below the soil surface. It was the most common destructive pest in rice of South East Asia in the past, but is presently causing damage to the rice in Thailand [9]. The main problem caused by this rice grasshopper species is on the leaves of young seedlings. This widespread species mainly inhabits rice cultivation in the low–lying grasslands, rice fields, and their surrounding banks. *O. chinensis* belongs to subfamily Oxyinae, family Acrididae under order Orthoptera. There are 18 species and six subspecies in this genus. Their difference in characters are based on eight morphological characters of the phallic complex according to Hollis's classification. Some authors reported that taxonomy of *Oxya* species could be determined using morphological and cytological characters [16]. In a previous study, comparisons of karyotypes and chromosome C-banding patterns were investigated in five *Oxya* species (*O. chinensis*, *O. shanghalensis*, *O.*

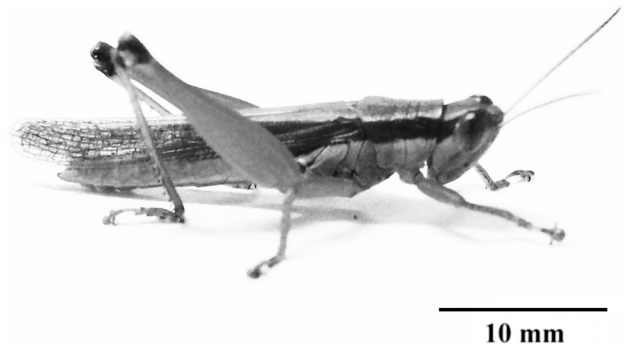

Fig. 1 General characteristic of a male small Chinese rice grasshopper (*O. chinensis*)

adentata, *O. hyla intricata* and *O. agavisa*) [15]. In 1994, Ma et al. [16] reported the cytogenetic studies of eight *Oxya* spp. (*O. chinensis*, *O. shanghalensis*, *O. adentata*, *O. agavisa*, *O. bicingula*, *O. hyla intricata*, *O.apicocingula* and *O. flave*) in China by C-banding. Recently, four *Oxya* species were analyzed by conventional and five differential-staining methods, all 4 species had a chromosome complement of $2n = 24$ (XX) in female and $2n = 23$ (XO) in male, with very similar karyotypes [23]. The insufficient cytogenetic information of rice grasshoppers in Thailand has inspired this study. This present work therefore attempts to describe the karyotype (chromosome number, morphology and chromosome size) and investigates the meiotic cell division of *O. chinensis* of Thailand. In the present study the basic data on cytogenetics obtained for this species will be additionally useful to the taxonomical characters for this genus.

Materials and methods

Ten adult males of *O. chinensis* used for this study were collected in the area of Khon Kaen province, Thailand. They were then taken to the laboratory. Cytological preparations were obtained from testes by cell suspension technique. Briefly, the testes were removed from grasshoppers after they were injected with 0.01% colchicine (in vivo) and kept for an hour. The testes were then gently minced in 0.075 M KCl in eppendorf tube and left to incubate for 30 min. The 0.075 M KCl was discarded from the supernatant after centrifugation at 1200 rpm for 8 min. Cells were then fixed by gradual addition of fresh cooled fixative (3 methanol:1 glacial acetic acid) to make up the volume to 2 ml before being centrifuged again at 1200 rpm for 8 min. The supernatant was then discarded. The fixation step was repeated until the supernatant appeared clear and the pellets were then mixed with 1 ml of fixative. The mixture was then dropped onto clean-cold glass slides by a micropipette, and the slides were air dried.

Conventional staining of the chromosomes in the dried slides was done using 10% Giemsa solution for 10 min. Detection of the NORs was carried out following the silver staining method of Howell and Black [11] with slight modification to reveal the presence of active rDNA clusters. The lengths of short arm (Ls) and long arm (Ll) of chromosomes were calculated for the length of total arm chromosome (LT, LT = Ls + Ll). Relative length (RL) and centromeric index (CI) were also calculated. The CI was computed to classify the types of chromosomes according to Chaiyasut [7]. All parameters were used in preparation of karyotypes and ideograms.

Results and discussion

Conventional staining

The *O. chinensis* had a diploid chromosome number of $2n = 23$ (XO) in male similar to the observations previously reported [12, 13, 15, 16, 23, 28]. The karyotype comprised of eight long (pair 1–4), eight medium (pair 5–8) and six short (pair 9–11) acrocentric minute short arm autosomes. The acrocentric X chromosome was approximately same in size to the medium autosomes (Fig. 2). The mean lengths of short arm chromosomes (Ls), long arm chromosomes (Ll), total chromosomes (LT) and the relative length (RL), centromeric index (CI) from 10 metaphases were shown in Table 1. The mean lengths of the chromosomes ranged from 23.33 to 5.86 μm with a total haploid length of 161.61 μm. The chromosome length and relative chromosome length were used to construct the standardized idiogram (Fig. 3). The idiogram clearly revealed the chromosomes to occur in different size groups of long, medium and short. The lengths of the four long pairs of chromosomes (1–4) ranged from 23.33 ± 5.52 to 15.88 ± 4.06 μm, the same for four medium pairs of chromosomes (5–8) ranged from 13.86 ± 3.06 to 11.08 ± 2.31 μm (including X) while the range of lengths found in three pairs of short chromosomes was from 8.36 ± 1.59 to 5.86 ± 0.99 μm (±SD data not shown in the table). A number of cytogenetic studies in tribe Oxyini, genus *Oxya* were reported earlier. In this study, some chromosomes were found to be different in morphological characters when compared with the data obtained in previous studies [15, 23] (Table 2).

Silver staining

This technique is useful to observe the secondary constriction (SC) of acridid grasshopper chromosomes, as described by Fox and Santos [10] in *Schistocerca gregarina* and *Locusta migratoria*. In this study with *O.*

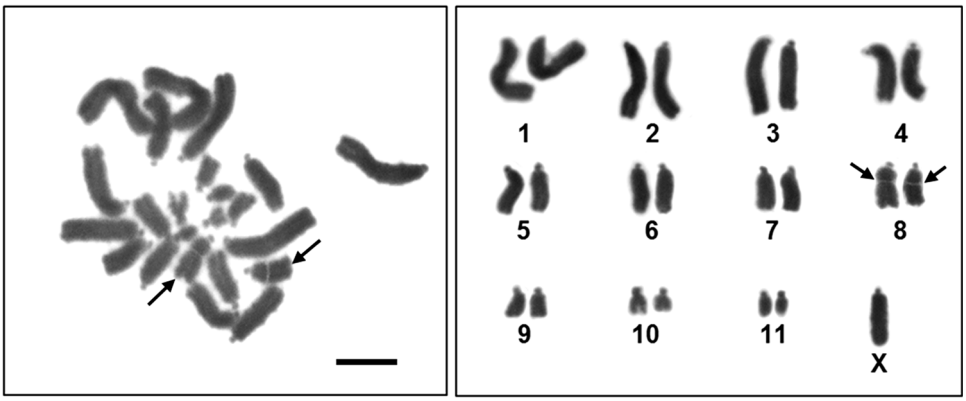

Fig. 2 Metaphase chromosome plate and karyotype of male *O. chinensis*, $2n = 23$ (XO) by conventional staining technique. The *arrows* indicate secondary constriction (*scale bars* 10 μm)

Table 1 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosomes (LT), relative length (RL), centromeric index (CI) from 10 metaphases of Small Chinese Rice grasshopper (*Oxya chinensis*), $2n$ (diploid) = 23, XO (male)

Chro.	Ls	Ll	LT	CI ± SD	RL ± SD	Type	Size
1	2.01	21.32	23.33	0.90 ± 0.02	0.145 ± 0.01	a	L
2	1.98	18.91	20.90	0.90 ± 0.02	0.130 ± 0.01	a	L
3	2.09	15.75	17.84	0.89 ± 0.02	0.111 ± 0.01	a	L
4	2.13	13.75	15.88	0.86 ± 0.03	0.098 ± 0.01	a	L
5	2.21	11.68	13.89	0.83 ± 0.04	0.087 ± 0.01	a	M
6	2.16	10.12	12.27	0.82 ± 0.04	0.076 ± 0.00	a	M
7	2.04	9.68	11.72	0.82 ± 0.04	0.074 ± 0.01	a	M
8	2.03	9.05	11.08	0.81 ± 0.04	0.069 ± 0.01	a	M
9	2.05	6.31	8.36	0.75 ± 0.04	0.052 ± 0.00	a	L
10	2.14	5.04	7.18	0.70 ± 0.06	0.045 ± 0.00	a	L
11	1.84	4.02	5.86	0.68 ± 0.05	0.037 ± 0.01	a	L
X	2.32	14.79	13.31	0.86 ± 0.03	0.076 ± 0.05	a	M

Chro. chromosome pair, *a* acrocentric, *L* large, *M* medium, *S* small

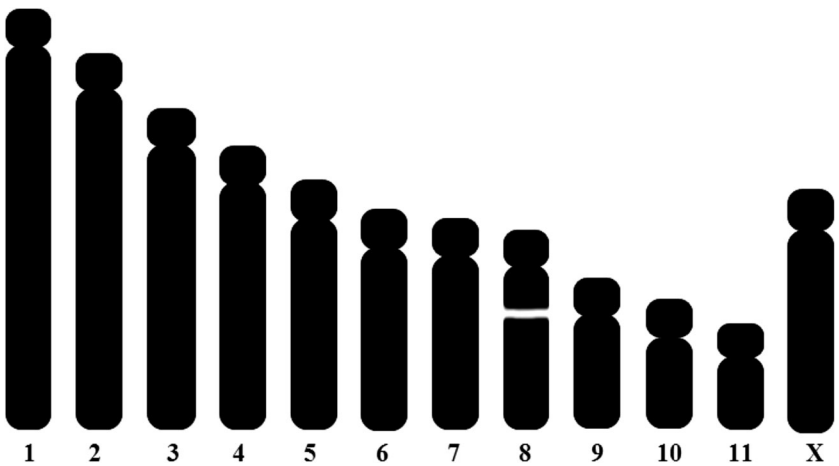

Fig. 3 Idiogram showing chromosome lengths and shapes of the *O. chinensis*, $2n = 23$ (XO) by conventional staining technique (*arrow* indicate secondary constriction)

chinensis, the SC that was found on chromosome 8 by conventional staining (Fig. 2) did not correspond to NOR at all by silver staining technique (Fig. 4). In 1986, Suja et al. [25] studied an acridid species *Psophus stridulus* and showed the sixth type of SC that corresponded to potential NOR region. Although 'type 6' was found as inconstant constriction, the SC of chromosome 8 in *Oxya* always appeared. Consequently, it corresponds, in all possibility, to the 'type 3' described by King [14] that is positive for C-banding and negative for silver staining.

Here, we found that NORs of *O. chinensis* were most probably present at the telomere of all minute short arms, the numbers of which were same as those of NORs but different in position as found by Yoshimura et al. [30]

Table 2 Review of cytogenetic reports of tribe Oxyini (Orthotera: Acrididae)

Species	2*n*	Karyotype formula	Sex mechanism	CM	NORs	Locality	References
Caryanda cultricerca	23	$3L + 4M + 4S + X$	XO, XX	t	–	China	Qing et al. [18]
C. amplexicerca	23	$4L + 4M + 3S + X$	XO, XX	t	–	China	Qing et al. [18]
Oxya chinensis	23	$2L + 6M + 3S + X$	XO, XX	a	–	China	Ma and Zheng [15]
	23	$2L + 7M + 2S + X$	XO, XX	t	–	Japan	Yoshimura et al. [30]
	23	–	XO, XX	a	–	China	Ma et al. [16]
	23	$4L + 4M + 3S + X$	XO, XX	a	23	Thailand	Present study
O. shanghalensis	23	$2L + 6M + 3S + X$	XO, XX	a	–	China	Ma and Zheng [15]
	23	–	XO, XX	a	–	China	Ma et al. [16]
O. adentata	23	$2L + 6M + 3S + X$	XO, XX			China	Ma and Zheng [15]
O. hyla intricatea	23	–	XO, XX	a	–	China	Ma et al. [16]
	23	$2L + 6M + 3S + X$	XO, XX	a	–	China	Ma and Zheng [15]
	23	$2L + 7M + 2S + X$	XO, XX	a	23	Japan	Yoshimura et al. [21]
	23	–	XO, XX	a	–	China	Ma et al. [16]
O. agavisa	23	$2L + 6M + 3S + X$	XO, XX	a	–	China	Ma and Zheng [15]
	23	–	XO, XX	a	–	China	Ma et al. [16]
O. bicingula	23	–	XO, XX	a	–	China	Ma et al. [16]
O. apicocingula	23	–	XO, XX	a	–	China	Ma et al. [16]
O. flave	23	–	XO, XX	a	–	China	Ma et al. [16]
O. japonica japonica	23	$2L + 7M + 2S + X$	XO, XX	a	23	Japan	Yoshimura et al. [30]
O. yezoensis	23	$2L + 7M + 2S + X$	XO, XX	t	23	Japan	Yoshimura et al. [30]
O. velox	23	$3L + 6M + 2S + X$	XO, XX	t	–	India	Fox et al. [10]

2n diploid chromosome, *L* Large chromosome, *M* medium chromosome, *S* small chromosome, *CM* chromosome morphology, *t* telocentric, *a* acrocentric, – not available

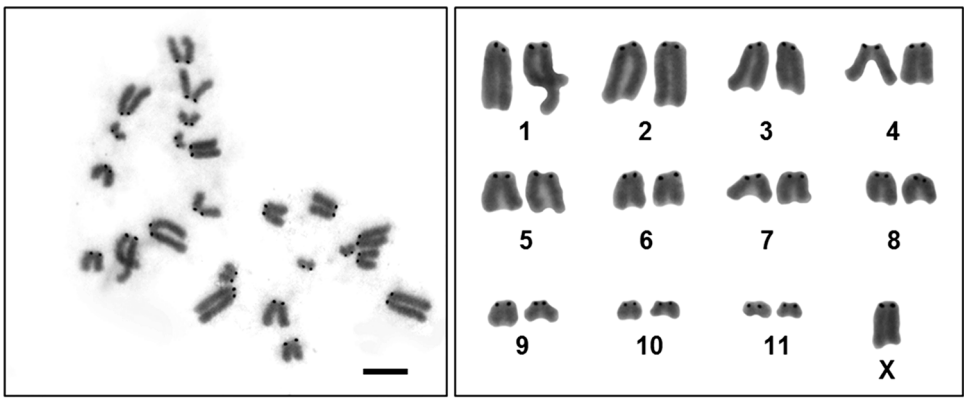

Fig. 4 Metaphase chromosome plate and karyotype of male *O. chinensis*, $2n = 23$ (XO) by silver staining technique (*scale bars* 10 μm)

(Fig. 4), the idiogram was also shown in Fig. 5. The results obtained here are different from most of the studies reported to date on this aspect of rice grasshopper species where only a few chromosome pairs, five pairs at the most, are involved in NORs [3–6, 20, 25, 29]. It is generally accepted that the maximum number of nucleoli per nucleus corresponds to the number of NORs [26]. Numerous silver deposits were detected in silver-stained Prophase nucleus of *O. chinenesis* (Fig. 6a). Therefore, it is likely that the silver deposits on all the telomere in this species reflect the presence of active NORs. However, none of the centromeres of pachytene chromosomes had silver deposit when silver staining was performed using male meiotic cells of *O. chinensis formosana* [30]. Such inconsistency in the NORs distribution between mitotic and meiotic chromosomes was reported in an acridid species *Pamphagus ortolanii* [17]. Mansueto and Vitturi [17] interpreted the reason as the difference in transcriptional activity of

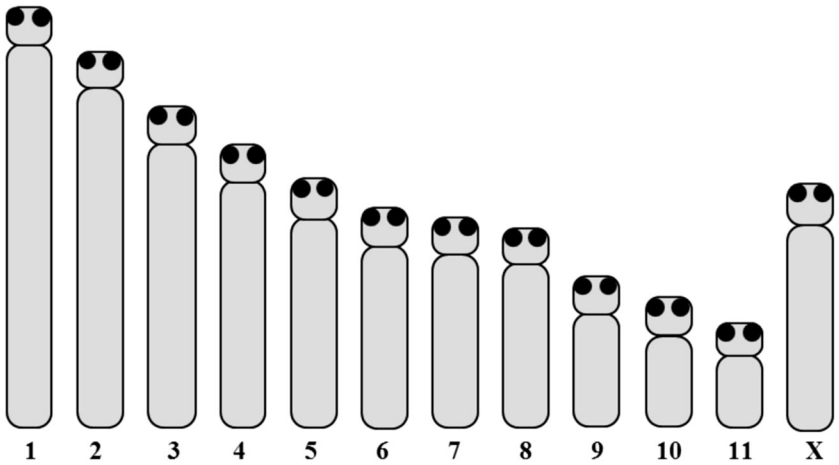

Fig. 5 Idiogram showing chromosome lengths and shapes of the *O. chinensis*, $2n = 23$ (XO) by silver staining technique

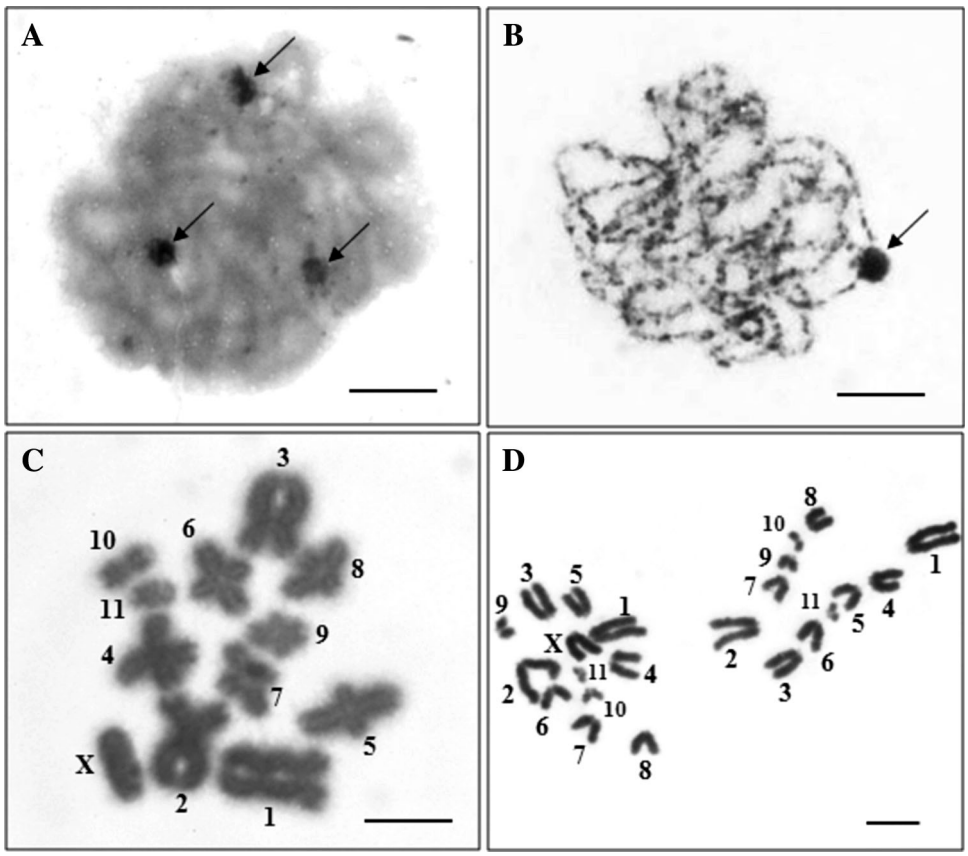

Fig. 6 Meiotic studies of *O. chinensis*. Leptotene stage by silver staining (**a**), pachytene stage (**b**), Metaphase I stage (**c**) and metaphase II stage (**d**) by conventional staining. The *arrows* indicate nucleolar organizer regions/NORs (*scale bars* 10 μm)

ribosomal DNA. On the other hand, it is also known that the $AgNO_3$ treatment is capable of depositing silver grains on the kinetochores of the highly condensed grasshopper chromosomes, depending on the treatment condition [18]. Yoshimura et al. [30] suggested that some of the silver deposits on the centromeres of *Oxya* chromosomes might be unrelated to the presence of active NORs rather reflecting silver–stained kinetochores. FISH analysis with ribosomal DNA probes is necessary to clear that telomeric NORs function as NORs in this species.

In this study, the chromosome complement and NORs of *O. chinensis* have been described for the first time in Thailand that have 2n = 23 acrocentrics with minute short arms in male individuals. Previous reports reveal that most species of the family Acrididae have 2n = 23 (XO) chromosomes in males [1, 2, 8, 19, 22–24, 27]. This suggests that the 2n = 23 acrocentric chromosomes is the common karyotype pattern for this family. So the short horn grasshoppers of different regions are showing cytogenetic uniformity regarding chromosome number and sex determining mechanism [21].

Meiotic study

The present study on meiotic cell division of *O. chinensis* found that during interphase, nuclelolus could be clearly seen while chromatins were absent. In prohase I, we found that cells had the distinctness of the observable leptotene (initiation of chromosome shrinking), zygotene (initiation of chromosome synapsis), pachytene (completion of chromosome synapsis), diplotene (chiasma and crossing over) and diakinesis (terminalization). In pachytene, we found that the NORs could be seen only in one region when performed by conventional staining (Fig. 6b) while three NOR regions were found by silver staining technique (Fig. 6a). According to Carvalho [6] impregnation with $AgNO_3$ was found in active nucleolar organizing regions when he analysed NORs in *Ommexecha virens* and *Descampsacris serrulatum*. In the Metaphase I of *O. chinensis* by conventional staining, the homologous chromosomes showed synapsis in 11 bivalents and one univalent X chromosome (Fig. 6c). Moreover, conventional staining in Metaphase II also showed the normal meiotic behaviour (Fig. 6d). It is confirmed that this species had the diploid number $2n = 23$ (XO) in male similar to previous reports (Table 2).

In conclusion, this study provides the first cytological details for *O. chinensis* of Thailand. The results support that the karyotype of tribe Oxyini are conserved among several other species. However, the chromosomal morphology may be slightly different depending on populations of *O. chinensis* present in different countries.

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