C-Banding Karyotypes of Two *Caryanda* (Orthoptera: Catantopidae) Species with Short Wings from China

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C-BANDING KARYOTYPES OF TWO *CARYANDA* (ORTHOPTERA: CATANTOPIDAE) SPECIES WITH SHORT WINGS FROM CHINA[1]

Liu Qing,[2,3] Ou Xiaohong,[3,*] and Ge Hongjie[2]

ABSTRACT: Chromosome C-band karyotypes, heterochromatin content and chromosome chiasma during meiotic prophase of two short-winged locust species *Caryanda cultricerca* and *Caryanda amplexicerca* (Orthoptera, Catantopidae) were studied. Results indicated that both species had a diploid chromosome number of 2n (♂) = 23 and a XO sex-determining mechanism. All chromosomes were telocentric except the S_9 of *C. cultricerca*. The autosomes can be classified into large, medium and short groups. In *C. cultricerca* there were 19 C-bands while *C. cultricerca* had 16 C-bands; both species had an approximately equal value in the total of heterochromatic content (21.91% vs. 21.58%). As for the chromosome behavior in meiosis, 1 chiasmata and 2 chiasmata types occurred most frequently in both species during meiotic prophase. Moreover, the differences in chromosome karyotype, characteristics of sex-chromosome and their significance in adaptation and differentiation of these two species are discussed.

KEY WORDS: Chromosome, C-banding karyotype, chiasma, *Caryanda cultricerca*, *Caryanda amplexicerca*

INTRODUCTION

There are 430 grasshopper species in the family Catantopidae in southern China, and nearly one third of the long-winged species have been studied for their chromosome characteristics (Zhao et al., 2010). *Caryanda cultricerca* Ou, Liu & Zheng, 2007 and *C. amplexicerca* Ou, Liu & Zheng, 2007 are two short-winged species occurring only in Yunnan, southern China. In 2007, Ou et al. first undertook morphological studies of these two species (Ou et al., 2007). In this paper we present the results of karyological analysis, using C-banding techniques, of the two species in order to find possible cytotaxonomic differences between them.

MATERIALS AND METHODS

We studied the following material from Yunnan Province, southern China: twelve males of *C. cultricerca* collected in Lingcang, and eleven males of *C. amplexicerca* from Gejiu (Table 1).

Testes were the tissues used for the experiment. Male specimens were injected with 4 to 6 µl 0.05% colchicine according to size and held for 6-8 hours. The tissues were then dissected and put in distilled water for 5-10 minutes, then fixed in a mixture of methanol and glacial acetic acid (3:1 v/v) for about 8-12 hours. The tissues were then transferred to 70% ethanol and stored at 4°C until use. Subsequently they were squashed in 60% glacial acetic acid, frozen in liquid nitrogen, the cover slip removed with a razor blade, and the slides then air-dried at room temperature for 2-

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5 days (Yao et al., 1995). C-banding was induced by BSG method (Webb et al., 1978). The preparations were examined under a Nikon ECLIPSE E800 microscope. About 5 to 10 clear and well-distributed metaphase plates were photographed. Some karyotypical characters, absolute length of chromosomes and C-bands length of each species were measured, other parameters of chromosomes such as relative length (RL), heterochromatic content (HC) and total of heterochromatic content (THC) of each species were calculated. Chromosomes were classified according to Levan et al. (1964).

Table1. Origin of the two *Caryanda* species samples

Species	Date	Location and Altitude	Collector	Quantity
Caryanda cultricerca	Aug. 2006	Dedangtown, Lincang (2060 m)	Q. Liu	12(♂)
Caryanda amplexicerca	Sept. 2006	Kafang town, Ge jiu(2100 m)	Q. Liu	11(♂)

RESULTS

Caryanda cultricerca

This species has a diploid chromosome number of 2n (♂) = 23 with a fundamental number = 25 and an XO sex-determining mechanism. The autosomes can be divided into three size groups according to their relative lengths: 3 pairs of large chromosomes (L_{1-3}), 4 pairs of medium chromosomes (M_{4-7}), 4 pairs of small chromosomes (S_{8-11}). The X-chromosome is the second element in size. All chromosomes are telocentric except S_9, which is a metacentric chromosome. The karyotype formula is 2n (♂) = 3L+4M+4S+X (Fig. 1).

The C-banding pattern of this species is characterized by the presence of centromeric C-bands in all chromosome pairs. Moreover, there are six additional terminal C-bands respectively in pairs L_3, M_5, M_6, S_8, S_{10} and the X-chromosome (Fig. 2). The total of heterochromatic content (THC) in this species is 21.91%; the highest content is 66.94% in the pair S_9, then 55.38% in S_{10} and the lowest one is 5.26% in the largest pair L_1 (Table 2).

The occurrence of chiasmata between nonsister chromatids of homologus chromosomes is a specific chromosome behavior in meiosis. Twelve cells in diplotene of prophase of this species were observed. The data showed that non-chiasmata type occurred 30 times, 2 chiasmata occurred most frequently at 51 times, accounting for 50.50% of the total chiasmata frequency, then 1 chiasmata (27 times, 26.73%) and 3 chiasmata (21 times, 20.79%), and 4 chiasmata occurred least, only 2 times and accounting for 1.98% of the total chiasmata frequency.

Caryanda amplexicerca

The karyotype of this species consists of 2n (♂) = 23 telocentric chromosomes with a fundamental number = 23 and an XO sex-determining mechanism. The autosome size groups are similar to those of *C. cultricerca* and also can be divided into three size groups according to the relative lengths: 4 pairs of large (L_{1-4}), 4 pairs of medium (M_{5-8}) and 3 pairs of short (S_{9-11}). The X-chromosome is the fifth element in size. The karyotype formula is 2n (♂) = 4L+4M+3S+X (Fig. 3).

Table 2. Chromosome data of *Caryanda cultricerca* in mitosis metaphase.

Number	groups	Absolute length (μm)	Relative length (%)	C-bands length (μm)		Heterochromatic content (%)	Arm index	Chromosome type
				Centromeric bands	Terminal bands			
1	L_1	15.22	20.16	0.80		5.26	17.13	T
2	L_2	10.85	14.37	0.88		8.11	9.38	T
3	L_3	8.40	11.13	0.78	2.03	33.45	∞	T
4	M_4	6.16	8.16	1.54		25.00	∞	T
5	M_5	5.99	7.94	0.80	1.33	35.56	∞	T
6	M_6	4.48	5.94	0.58	0.70	28.57	∞	T
7	M_7	4.06	5.38	0.70		17.24	∞	T
8	S_8	3.15	4.17	0.53	0.94	46.67	∞	T
9	S_9	2.45	3.25	0.50	0.44	66.94	1.31	M
10	S_{10}	1.95	2.58	0.46	0.62	55.38	∞	T
11	S_{11}	1.20	1.59	0.32		26.67	∞	T
X	X	11.57	15.33	1.12	1.47	22.39	10.02	T
Total		75.48	—	16.54		21.91*	—	—

*Total of heterochromatic content (%) = Total of C-bands length (16.54)/Total of absolute length of chromosome set (75.48)

b l e 2a Gh r oms mdms 2at l fl ami *Caryanda amplexicerca*apas nfmdrdas 2fl *r l d2a

Nus e2o	1omu*d	Aedmuf2a 2p1 fr (µs)	R2 1 fnv2a 2p1 fr (%)	h gel pt da2p1 fr (µs)		H2f2on(r oms 1 fn(a (mpf2pf (%)	Aos rpt 2x	h r oms mdms 2a fy*2
				h 2pfoms 2or(a el pt d	b2os rpl a el pt d			
T	L_T	T0C3	T3CT.	TC56	TCT	00C98	∞	f
0	L_0	T7C65	T. C09	TC74	7C4T	T6C97	∞	f
.	$L_.$	T7CT3	T0C.	7C66	TC09	07C77	∞	f
5	L_5	8C99	TTC80	7C84		T7CT5	∞	f
3	M_3	9C55	6C83	TC78		T9C8.	∞	f
9	M_9	3C83	6C3	TC65	TC89	90CT4	∞	f
6	M_6	3C.	9C4.	7C44		T3C8T	∞	f
4	M_4	5C87	9C73	TC73		0TC5.	∞	f
8	S_8	. CT0	. C43	7C66		05C94	∞	f
T7	S_{T7}	0C47	. C59	7C9.		00C37	∞	f
TT	S_{TT}	TC56	TC40	7C39		. 4CT7	∞	f
X	X	6C84	8C43	TCT0		T5C75	∞	f
bmfl		47C88	—	T6C54		0TC34c	—	—

cbmfl amiar 2f2on(r oms 1 fn(a(mpf2pf2%) =a- abmfl amiah gel pt da2p1 fr a%T6C54=/bmfl amial edmuf2a 2p1 fr amia(r oms mdms 2ad2fa%47C88=aa

The C-banding pattern of this species indicates the existence of centromeric C-bands in all chromosomes and four additional terminal C-bands in the pairs L_1, L_2, L_3, M_6 (Fig. 4). The total heterochromatic content is 21.58%; the highest content is 62.18% in M_6, then 38.10% in S_{11} and the lowest is 10.14% in L_4 (Table 3).

Table 4. Comparison of *C. cultricerca* and *C. amplexicerca* chromosome characteristics

Species	Karyotype Formula	Chromo-some Number	THC %	C-bands Number	Non-Chias-mata	1 Chias-mata	2 Chias-mata	3 Chias-mata
C. *cultricerca* (12 cells)	3L+4M+4S+X		21.91	19	30	27	51	21
C. *amplexicerca* (7 cells)	4L+4M+3S+X	23	21.58	16	19	20	34	8

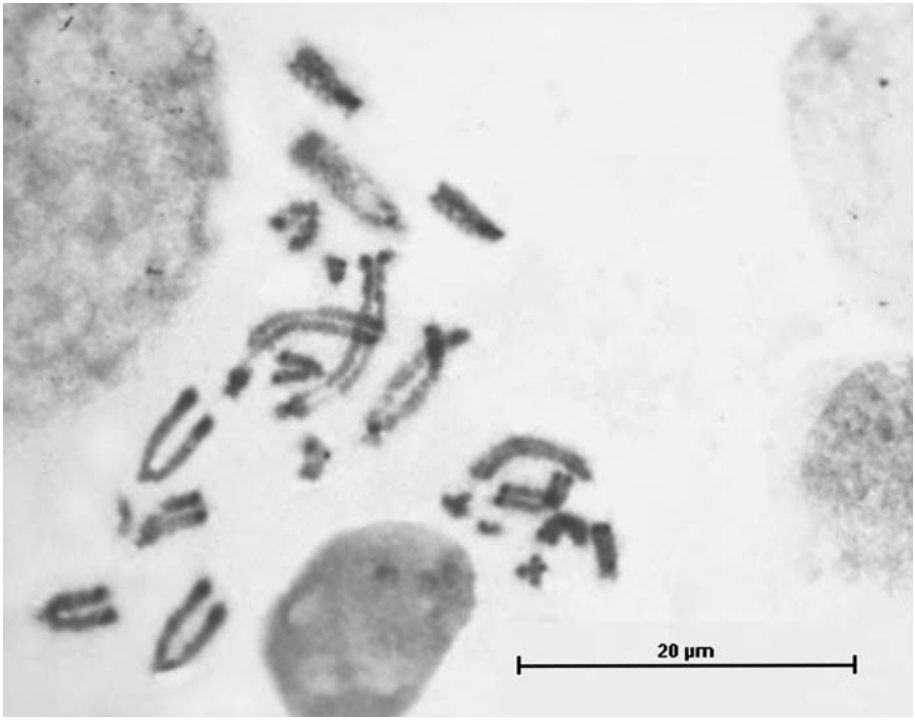

Fig. 1. C-banding karyotype of *Caryanda cultricerca*

Table 5. Comparison of the sex chromosome of *C. cultricerca* and *C. amplexicerca*

Species	Rank	Type	X	HC(%)
C. cultricerca	2	L	2	22.39
C. amplexicerca	5	M	1	14.04

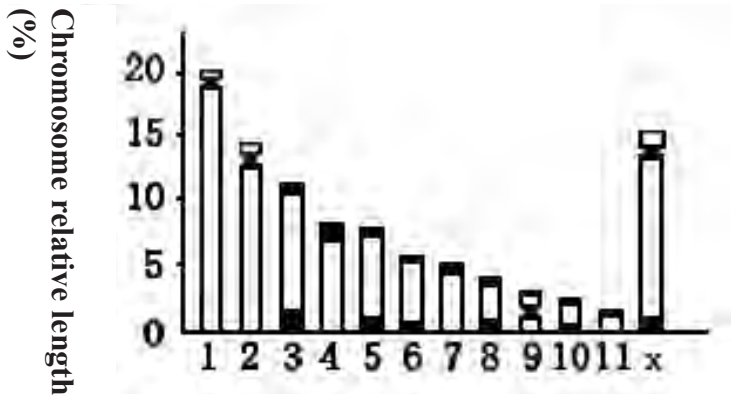

Fig. 2. Idiogram of *Caryanda cultricerca*

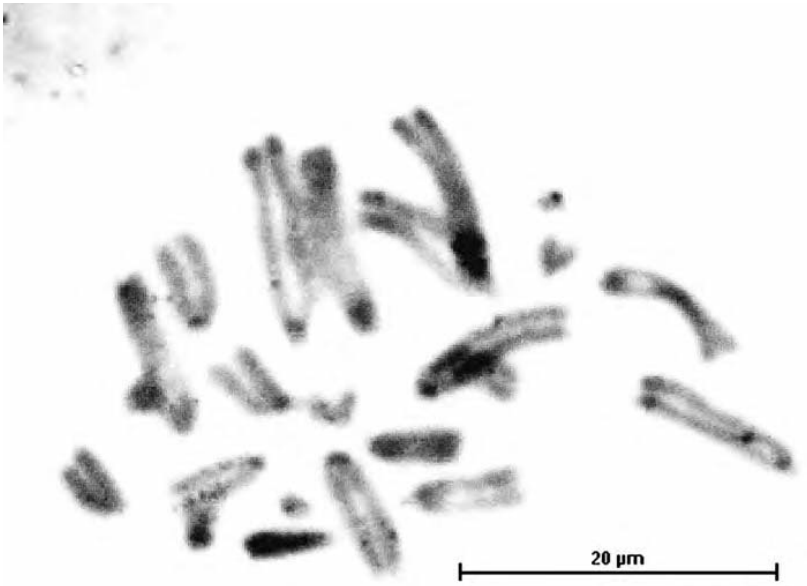

Fig. 3. C-bands karyotype of *Caryanda amplexicerca*

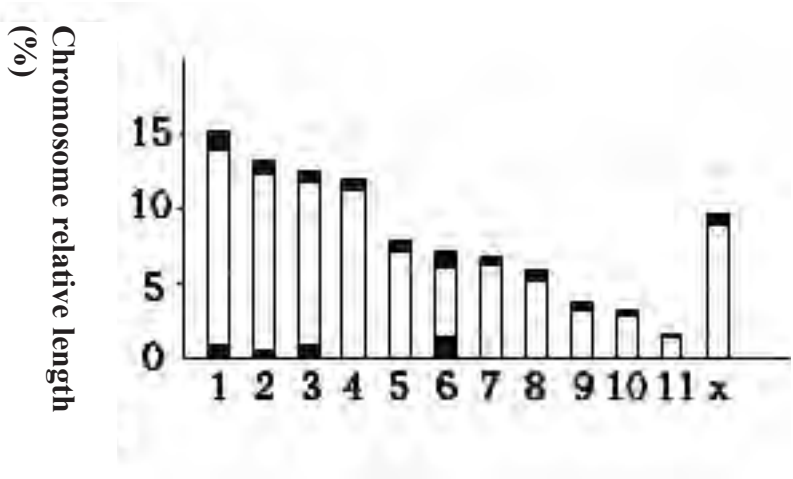

Fig. 4. Idiogram of *Caryanda amplexicerca*.

In this species, seven cells in diplotene stage of prophase were observed; non-chiasmata type occurred 19 times, 2 chiasmata occurred most frequently at 34 times, accounting for 54.84% of the total chiasmata frequency, then 1 chiasmata (20 times, 32.26%) and 3 chiasmata (8 times, 12.90%). Unlike in *C. cultricerca*, there were no 4 chiasmata type in this species.

DISCUSSION

Chromosome characteristics of the two *Caryanda* species

The two species of *Caryanda* analyzed in this paper present similarities in chromosome characteristics and behaviors. Both species have the same chromosome number of 2n (♂) = 23 and an XO sex-determining mechanism. Furthermore, the autosome size groups are also similar in the two species and can be divided into three size groups according to their relative lengths: large, medium and short. There are 19 and 16 C-bands in *C. cultricerca* (♂) and *C. amplexicerca* (♂), respectively, which is not much difference. At the same time, both species have approximately the same value in the total of heterochromatic content (21.91% vs. 21.58%). As for the chromosome behavior in meiosis, 1 chiasmata and 2 chiasmata types occurred most frequently in both species during meiotic prophase. Genomes of both species are all telocentric except the S9 of *C. cultricerca* (Table 4).

However, *C. cultricerca* and *C. amplexicerca* still show some differences in karyotype formula since in the former species there are 3 pairs of large chromosomes and 4 pairs of short chromosomes, while *C. amplexicerca* has the opposite situation. Moreover, both species show very different characteristics of the sex-chromosome as discussed below.

Characteristics of the sex-chromosome

The sex-chromosome of *C. cultricerca* is a large chromosome in its relative length (RL = 15.33) and has a heterochromatin content (HC) of 22.39%. The sex-chromosome of *C. amplexicerca* is a medium-sized chromosome with a RL value of 9.85 and has a heterochromatin content of 14.04% (Table 5).

The family-level chromosome characteristics are generally embodied in the karyotype (number of chromosomes). The genus-level characteristics are reflected in the silver-staining nucleolus organizer region (NOR) and landmark C-bands karyotype, while size of terminal-bands, centromere-bands and heterochromatin content characterizes species (Bugrov et al., 1994; Ma et al., 2000; Ma and Guo, 2001; Ma et al., 2002). Although *C. cultricerca* and *C. amplexicerca* have the same XO sex-determining mechanism, the size of their X chromosomes and the heterochromatin contents are significantly different, which, in addition to morphological characteristics, can be used to differentiate these two species.

Adaptation and differentiation

C-bands contain the structure of chromosome heterochromatin regions. It has been confirmed that C-band is highly repetitive with AT and CG sequences, including satellite DNA sequence. C-bands are located in the telomere and centromere areas as well as in the secondary chromosome constriction arm. Because it is relatively stable, the C-band can be used to classify genera, species and subspecies. C-bands are not only involved in the formation and spatial arrangement of heterochromatin, but also lead to the distribution of chromatin as DNA of the transposable element, which may cause genetic variation (Ma et al., 2000; Zhai et al., 2000). In many groups of organisms, changes in the amount of heterochromatin seem to have played a fundamental role in speciation (Nagl, 1978). Therefore, the number of C-bands and THC can be considered as an indicator for intensity of genetic differentiation (Zhai et al., 2000). According to data in Table 5, *C. cultricerca* seems to be slightly more genetically differentiated than *C. amplexicerca*. However, it is necessary to study more samples of these two species to draw a firm conclusion.

Yunnanites coriacea Uarov, also a short-winged locust species widely distributed in the highlands of Yunnan, Guizhou and Sichuan Provinces, has evolved to adapt to different habitats. In contrast, *C. cultricerca* and *C. amplexicerca* are found only in a narrow range at low-elevation tropical areas near Gejiu and Lincang Townships, Yunnan Province, where food sources and suitable oviposition sites are readily available. They are adapted to the local habitat and do not need to fly far for food, and this life habit has resulted in shortened wings. Wing shortening becomes a further barrier for gene flow between different species, which appears to be the mechanism of species differentiation.

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