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Chromosome studies on the mitten crabs *Eriocheir japonica* and *E. sinensis*

TAI-HUNG LEE,* NANAMI NAITOH AND FUMIO YAMAZAKI

Graduate School of Fisheries Science, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan

ABSTRACT: In recent years, there has been debate from various perspectives on the question of whether the mitten crabs *Eriocheir japonica and E. sinensis* should be considered as two different species. It has been reported that the chromosome numbers of these two crabs differ from each other; for *E. japonica* it is 2n = 148, n = 74, whereas for *E. sinensis* it is 2n = 146, n = 73. These data suggest that *E. japonica* and *E. sinensis* are different species. Nevertheless, the chromosome number of *E. japonica* was obtained in 1937 by the method of paraffin section. This classical gonad (testis) sectioning method is now known as an unreliable method for determining the number of chromosomes. In the present study the number of chromosomes in *E. japonica* and *E. sinensis* is re-examined using the air-drying method. The results show that *E. japonica and E. sinensis* have the same number of chromosomes (2n = 146, n = 73), which means that the number of chromosomes cannot be used as a criterion to distinguish these two species. Even though observation of the chromosomes under the scanning electron microscope was attempted, it was not possible to perform karyotype analysis in the two species because centromeres could not be identified in some of the chromosomes.

KEY WORDS: **chromosome, crab, *Eriocheir japonica*, *Eriocheir sinensis*.**

INTRODUCTION

The two species of mitten crabs, *Eriocheir sinensis* and *E. japonica*, are commercially important aquatic animals in China. *Eriocheir sinensis* is distributed in northern and eastern China, while *E. japonica* has a broad distribution from eastern Korea and Japan to southern China.[1]

In recent years, there has been debate from various perspectives on the question of whether the mitten crabs *E. japonica and E. sinensis* should be considered as two different species. Based on the fact that *E. japonca* shares many apomorphic and morphological characters with *E. sinensis*, Dai claimed that *E. japonica* was probably only a subspecies of *E. sinensis*.[2] Li *et al.* compared the morphometric and biochemical variations of *E. sinensis* and *E. japonica* populations.[3] They suggested that the morphological differences among the mitten crabs in southern (i.e. *E. japonica*) and northern (i.e. *E. sinensis*) China might be eco-phenotypic and synonymized *E. sinensis* with *E. japonica*. Furthermore, it has been shown that *E. sinensis* and *E. japonica* can mate and produce hybrid offspring.[4,5]

However, in other studies based on morphometric, isozyme, rapid amplified polymorphic DNA analysis and cytochrome oxidase subunit I sequences analysis distinct differences were detected between the southern (i.e. *E. japonica*) and northern (i.e. *E. sinensis*) populations.[6–11]

Cytogenetical studies provide us with fundamental information for examining the taxonomic status of these two species. It has been reported that the number of chromosomes in *E. japonica* differs from that in *E. sinensis*. In *E. japonica*, it is 2n = 148, n = 74,[12] whereas in *E. sinensis* it is 2n = 146, n = 73.[13] These data suggest that *E. japonica* and *E. sinensis* are different species.

Nevertheless, the chromosome number of *E. japonica* was obtained by the method of paraffin section in 1937. This classical gonad (testis) sectioning method is now known to be an unreliable method for determining the number of chromosomes.

In the present study we re-examine the number of chromosomes in *E. japonica* and *E. sinensis*

*Corresponding author: Tel. 81-138-40-5590. Fax: 81-138-40-5590. Email: thlee@fish.hokudai.ac.jp
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using a reliable method (i.e. the air-drying method).[14–16]

MATERIALS AND METHODS

Adult specimens of Japanese mitten crabs were collected from Shizuoka Prefecture (Hamanako Lake) in Japan, and specimens of Chinese mitten crabs were imported from Shanghai, China, both in October 1997. Based on their different taxonomic characters,[1,17] specimens from Japan and China were recognized as *E. japonica* and *E. sinensis*, respectively. The animals were reared in a closed circulating system (50% seawater) at 20°C.

The operations of the chromosome observations were processed as follows.

(1) Male adult crabs were injected with colchicine solution intramuscularly at 1 μg colchicine : 1 g body weight twice at an interval of 24 h.

(2) After a 48 h treatment of colchicine, the animals were dissected. Their testes were taken out, and pieces of testis tissues were placed in distilled water or 0.001% sodium citric acid solution for hypotonic treatment.

(3) Samples of testis tissues were then fixed in freshly prepared Carnoy solution (methanol : acetic acid, 1:1) and rinsed three times with Carnoy solution, each lasting 10 min, resuspending the tissues at each change.

(4) Fixed testis tissues were chopped into paste using a chopping machine[18] on a slide glass coated with silicone. The paste was filtered through a net (mesh size: 200 μm × 100 μm). The cells of the testes were then rinsed three times by centrifugal separation (1500 ×*g*, 5 min each time). Afterwards, the supernatant was decanted, new Carnoy solution was added, and the cells of the testis were resuspended.

(5) Two to three drops of the suspension of the cells were put on a slide glass and were inflamed immediately.

(6) After the slides were air-dried, they were stained for 15–20 min with Giemsa staining solution diluted with phosphate buffer (pH 6.8). The slides were then rinsed three times with distilled water within 1 min and then the slides were air-dried again.

(7) The symmetrically well-spread metaphase chromosome plates were observed and photomicrographed with a light microscope (Olympus BH-2; Olympus Optical, Tokyo, Japan).

(8) Slides were coated with platinum (8–9 nm thick) using an ion sputter (JEOL JFC-1100; JEOL, Tokyo, Japan) for scanning electron microscope (Hitachi S-2300; Hitachi, Tokyo, Japan) observations and photomicrography.

Table 1 *Eriocheir japonica* and *E. sinensis* MMC, CN, and percentage obtained from testes

	E. japonica		*E. sinensis*		
	MMC	%	MMC	%	CN
	0	0.00	1	1.19	65
	1	1.05	1	1.19	66
	0	0.00	2	2.38	67
	1	1.05	3	3.57	69
	3	3.16	2	2.38	70
	7	7.37	0	0.00	71
	10	10.53	4	4.76	72
	63	66.32	55	65.48	73†
	6	6.31	6	7.15	74
	4	4.21	5	5.95	75
	0	0.00	1	1.19	76
	0	0.00	3	3.57	77
	0	0.00	1	1.19	78
Total	95	100.00	84	100.00	

MMC, meiotic metaphase cells; CN, chromosome number.
† Modal haploid chromosome number (n = 73)

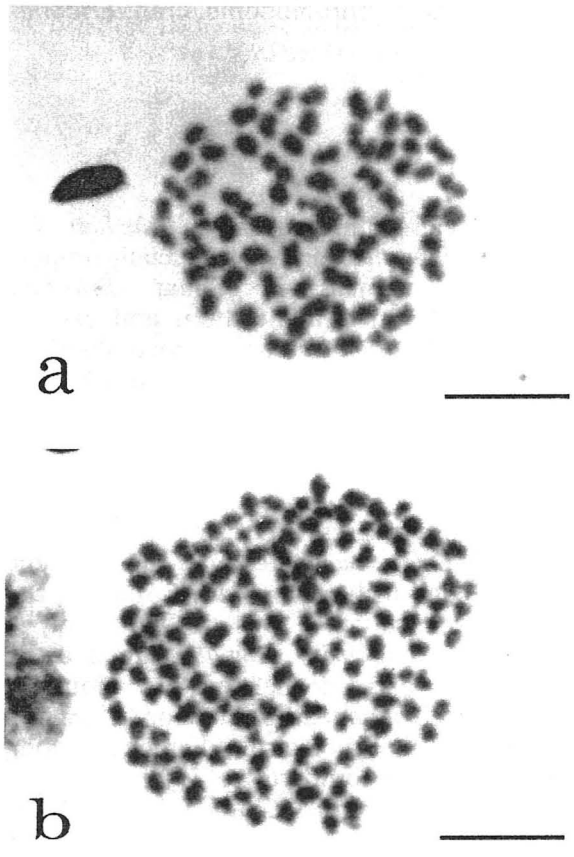

Fig. 1 Metaphase chromosomes obtained from testes of *Eriocheir japonica*. (a) Meiotic chromosomes; (b) mitotic chromosomes. Bars = 10 μm.

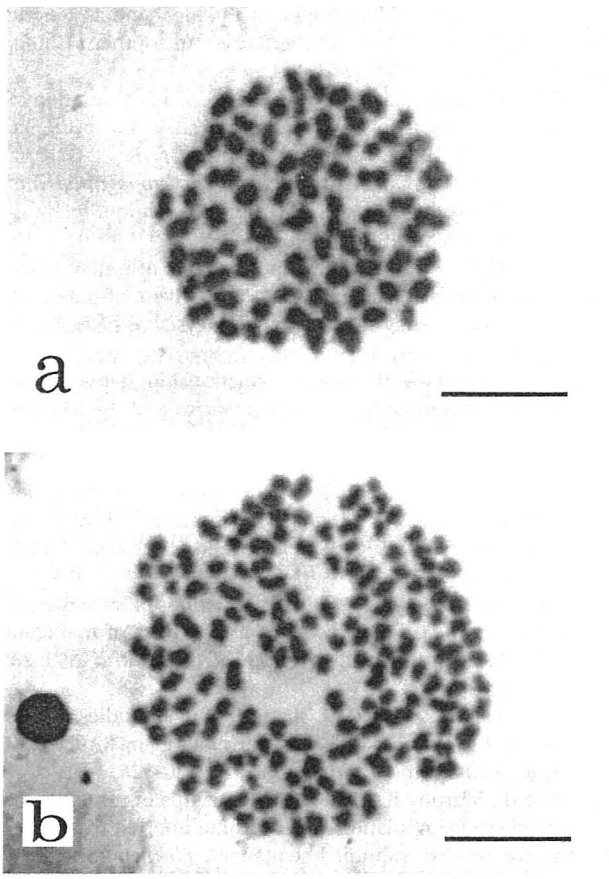

Fig. 2 Metaphase chromosomes obtained from testes of *Eriocheir sinensis*. (a) Meiotic chromosomes; (b) mitotic chromosomes. Bars = 10 μm.

Table 2 *Eriocheir japonica* and *E. sinensis* MMC, CN, and percentage obtained from testes

	E. japonica		*E. sinensis*		
	MMC	%	MMC	%	CN
	1	3.23	0	0.00	130
	1	3.23	0	0.00	136
	1	3.23	0	0.00	137
	1	3.23	0	0.00	138
	1	3.23	1	4.17	141
	1	3.23	0	0.00	142
	1	3.23	1	4.17	143
	1	3.23	2	8.33	144
	2	6.40	2	8.33	145
	18	58.07	15	62.50	146[†]
	1	3.23	2	8.33	149
	1	3.23	0	0.00	151
	1	3.23	0	0.00	152
	0	0.00	1	4.17	155
Total	31	100.00	24	100.00	

MMC, mitotic metaphase cells; CN, chromosome number.
[†] Modal diploid chromosome number (2n = 146).

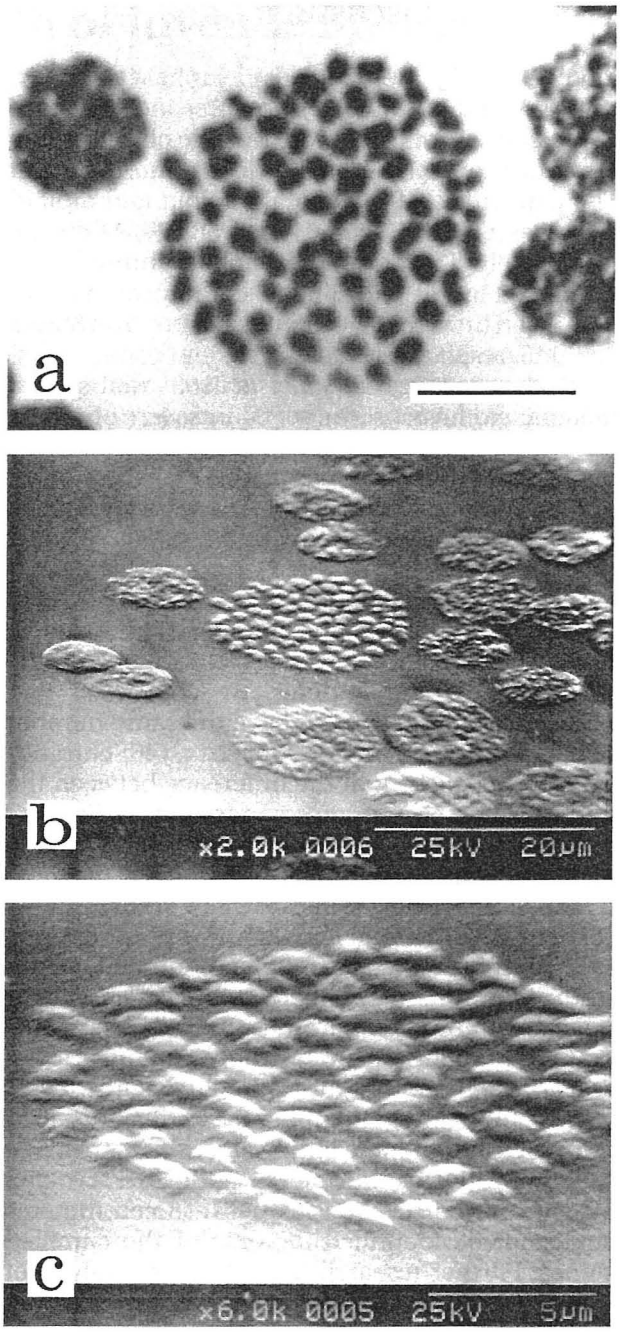

Fig. 3 Meiotic metaphase chromosomes obtained from testes of *Eriocheir japonica and* photomicrographed with a light microscope and a scanning electron microscope. In some of the chromosomes, centromeres could not be identified. (a) Light microscope (bar = 10 μm); (b) scanning electron microscope (bar = 20 μm); (c) scanning electron microscope (bar = 5 μm).

RESULTS AND DISCUSSION

Among the 126 well-spread metaphase chromosome set counts obtained from three individuals of *E. japonica*, 95 of them were meiotic chromosome sets and 31 of them were mitotic chromosome sets. The percentage of the modal haploid and diploid cells was 66.32% (Table 1) and 58.07% (Table 2), respectively. Therefore, the haploid chromosome number (Fig. 1a) and diploid chromosome number (Fig. 1b) were considered to be n = 73 and 2n = 146, respectively.

In the case of *E. sinensis*, 108 well-spread metaphase chromosome set counts were obtained from three individuals. Eighty-four of them were meiotic chromosome sets and 24 of them were mitotic chromosome sets. The percentage of the modal haploid and diploid cells was 65.48% (Table 1) and 62.50% (Table 2), respectively. Therefore, the haploid chromosome number (Fig. 2a) and diploid chromosome number (Fig. 2b) were considered to be n = 73 and 2n = 146, respectively.

Based on these findings, we concluded that *E. japonica* and *E. sinensis* have the same number of haploid (n = 73) and diploid (2n = 146) chromosomes. We believe that the difference between the number of chromosomes reported in Niiyama's study[12] and that found in the present study is due to the reliability of the methods rather than the materials being used, because the crabs used in Niiyama's study[12] and those in the present study were both from the same prefecture in Japan. Our study shows that *E. japonica and E. sinensis* have the same number of chromosomes, which means that the number of chromosomes cannot be used as a criterion to distinguish these two species.

Even though we attempted to observe the chromosomes under the scanning electron microscope (Fig. 3), we were not able to perform karyotype analysis in the two species because centromeres could not be identified in some of the chromosomes. Further research is needed for analysis of the karyotype.

REFERENCES

1. Sakai T. *Crabs of Japan and the Adjacent Seas.* Kodansha, Tokyo. 1976.
2. Dai A. A preliminarily cladistic analysis on *Eriocheir* (Crustacea: Decapoda). *Acta Zootaxon. Sinica* 1988; **13**: 22–26.
3. Li G, Shen Q, Xu Z. Morphometric and biochemical genetic variation of the mitten crab, *Eriocheir*, in southern China. *Aquaculture* 1993; **111**: 103–115.
4. Peng WH. Preliminary study on the problem of variation of *Eriocheir sinensis* in Zhujiang River Valley. *Inf. Fish. Sci. Tech.* 1986; **2**: 19–22.
5. Zhao NGN, Bao X, Zhang L. *Artificial Propagation and Aquaculture of the Mitten Crab.* Anhui Science and Technology, Hefei, Chian. 1988.
6. Li CH, Li SF. Phylogenesis of populations of mitten crabs (*Eriocheir sinensis, E. japonicus*) in six rivers of mainland China: morphology discriminant analysis. *J. Fish. China* 1999; **23**: 337–342.
7. Gao T, Watanabe S. Genetic relationship between the Japanese mitten crab, *Eriocheir japonica* and the Chinese mitten crab, *Eriocheir sinensis. Suisanzoshoku* 1998; **46**: 171–176.
8. Zhao JL, Li SF. Phylogenesis of populations of mitten crabs (*Eriocheir sinensis, E. japonicus*) in six rivers of mainland China: Biochemical genetic difference analysis. *J. Fish. China* 1999; **23**: 331–336.
9. Li SF, Zou SM. Phylogenesis of populations of mitten crabs (*Eriocheir sinensis, E. japonicus*) in six rivers of mainland China: RAPD fingerprinting marker. *J. Fish. China* 1999; **23**: 325–330.
10. Xie H, Lu RH, Xiang CM, Zhang J, Qiu T. Studies on the relationship of three kinds of mitten crabs using RAPD technique. *Acta Hydrobiol. Sinica* 1999; **23**: 120–126.
11. Zhao JL, Murphy RW, Li SF. Relationships of mitten crabs (*Eriocheir*) from inland rivers of China inferred from cytochrome oxidase subunit I sequences. *Biochem. Syst. Ecol.* 2002; **30**: 931–941.
12. Niiyama H. The problem of male heterogamety in the Decapod Crustacea, with special reference to the sex chromosomes in *Plagusia dentipes* de Haan and *Eriocheir japonicus* de Haan. *J. Fac. Sci. Hokkaido Imp. Univ. Ser. VI Zoology* 1937; **5**: 283–295.
13. Du NS. The chromosomes of the Chinese mitten-handed crab, *Eriocheir sinensis* (Crustacea, Decapoda). *Zool. Res.* 1986; **7**: 293–296.
14. Evans EP, Breckson G, Ford CE. An air-drying method for meiotic preparations from mammalian testes. *Cytogen* 1964; **3**: 289–294.
15. Yamazaki F, Onozato H, Arai K. The chopping method for obtaining permanent chromosome preparation from embryos of teleost fishes. *Nippon Suisan Gakkaishi* 1981; **47**: 963.
16. Murofushi M, Deguchi Y. A method for obtaining metaphase chromosomes from large shrimp-like crustaceans. *Rep. Mishima Res. Inst. Sci. Liv. Nihon Univ.* 1983; **6**: 31–34.
17. Guo JY, Ng NK, Dai A, Ng KLP. The taxonomy of three commercially important species of mitten crabs of the genus *Eriocheir* De Haan, 1835 (Crustacea: Decapoda: Brachyura: Grapsidae). *Raffles Bull. Zool.* 1997; **45**: 445–476.
18. Lee TH. Chopping machine for chromosome preparation. *IDEN (Tokyo)* 1990; **44**: 75–76.