KOREA STRAIT DELIMITING DISTRIBUTION OF DISTINCT KARYOMORPHS OF *CROCOTHEMIS SERVILIA* (DRURY) (ANISOPTERA: LIBELLULIDAE)

K. HIGASHI[1], C.E. LEE[2], H. KAYANO[3] and A. KAYANO[3]

[1] Department of Applied Biological Sciences, Faculty of Agriculture, Saga University,
Honjyo-machi 1, Saga 840-8502, Japan
e-mail: higashik@cc.saga-u.ac.jp
[2] Entomological Institute of Korean Peninsula, Hyomok-2Dong 615-2, Dong-ku,
Taegu 701-032, Republic of Korea
[3] Kujyu-Highland Institute of Biology, Kuju 4026, Kuju-machi, Naoiri-gun,
Oita 878-0201, Japan

In order to define the eastern limit of the distribution of *C. s. servilia* (2n♂ = 25, XO) and the western limit of *C. s. mariannae* (2n♂ = 24, neo-XY), the karyotypes were studied in specimens from Cheju island and the mainland of the Republic of Korea, and from the islands of North Kyushu, Japan. The dividing line between the 2 sspp. appears in the Korea Strait, between the Korean mainland and Tsushima island. The mechanism of maintaining a distinct karyomorph in each area of distribution is discussed in details.

INTRODUCTION

Crocothemis servilia occurs from the Middle East, through southern Asia, China, Australia, the Sundaic Archipelago to Japan and includes two distinct karyomorphs: one, 2n♂ = 25 with XO sex determining system, and the other, 2n♂ = 24 with neo--XY system. The former karyomorph was found in specimens from China, continental Asia, the Philippines and Taiwan, and the latter was found in specimens collected from Kyoto and Osaka in Japan (KIAUTA, 1983). On this basis, KIAUTA (1983) established a new subspecies, *C. servilia mariannae*, to separate the Japanese populations from those in other regions.

In a previous paper (HIGASHI & KAYANO, 1993), we reported that the distribution of the two karyomorphs of *C. servilia* split between the islands of Yakushima and Amamioshima. All the specimens from Amamioshima island and localities South of it had 2n♂ = 25 with XO, as found by KIAUTA (1983) in

specimens from Taiwan. All those from Yakushima island and localities North of it had 2n♂ = 24 with neo-XY, as found by KIAUTA (1983) in specimens from Kyoto and Osaka, Japan.

Taking the studies of KIAUTA (1983) and HIGASHI & KAYANO (1993) into account, INOUE & TANI (1999) and SUGIMURA et al. (1999) treated the populations of *C. servilia* distributed over the area from Amamioshima island to southern localities as *C. s . servilia* and the ones from Yakushima island and the localities North of it as *C. s. mariannae*.

In order to find the eastern limit of the distribution of *C. s. servilia* and the western limit of *C. s. mariannae*, we collected specimens from localities in Cheju island and the mainland of the Republic of Korea, and from islands of North Kyushu, Japan. The dividing line between the two subspecies was found to lie in the Korea Strait between the Korean main land and Tsushima island. The present paper also reports chromosomes of specimens collected from Thailand.

MATERIAL AND METHODS

Specimens of *Crocothemis servilia* used for chromosome preparation were collected from the following localities:

(1)   REPUBLIC OF KOREA: 1♂, Kangnung, Kangwondo, 13-VII-1994 (specimen No. Ka-1); − 3♂, Yongdok, Kyongsangpukdo, 12-VII-1994 (Nos Yo-1, 2 & 3); − 4♂, Kyongsan, Kyongsangpukdo, 14- -VII-1994 (Nos Ky-1, 2, 3 & 4); − 3♂, Sogwipo, Chejudo (Cheju-island), 5-VIII-1996 (Nos Ch-1, So-1 & 2).

(2)   JAPAN: 13♂, Kamiagatachou, Tsushima-island, Nagasaki pref. 13-VII-1992 (Nos Th-1, 2 & 3, Sh-1, 2, 3, 4, 5, 6, 7, 8, 9 & 10); − 3♂, Mitsushimachou in Tsushima island, Nagasaki pref. 14-VII-1992 (Nos Mt-1, 2 & 3); − 5♂, Gounourachou & Ishidachou, Iki island, Nagasaki pref., 15-VII-1992 (Nos Iki-1, 2, 3, 4 & 5); − 4♂, Fukue, Fukuejima island in Gotoretto Archipelago, Nagasaki pref., 31-VII-1997 (Nos Fu-1, 2, 3, 4 & 5).

(3)   THAILAND: 2♂, Chiang Mai (Chiang Mai Univ.), 1-X-1999 (Nos Chn-1 & 2), 8♂, Ban Bo Luang, 30- -IX & 1-X-1999 (Nos Bl-1, 2, 3, 4, 5, 6, 7 & 8).

The males were fixed with acetic alcohol (1:3) immediately after or within one day of capture. The specimens were transferred to 70% ethanol after two days of fixation or kept in the fixing solution and stored in a refrigerator.

To observe spermatogonial and primary spermatocyte chromosomes, testes were dissected out in 70 % ethanol or in the fixing solution and were stained for 2 to 3 hours with alcoholic hydrochloric acid-carmine (SNOW, 1953). Each testis was cut into pieces and each piece was made into a temporary squash preparation using 45% acetic acid. After dissection, the specimens were kept in 70% ethanol and stored in a refrigerator.

RESULTS AND DISCUSSION

Primary spermatocyte complements in specimens from the islands in North Kyushu, Japan (Tsushima, Iki, Fukuejima), showed twelve bivalents (n = 12) (Fig.1 A-C). This karyomorph, without an "*m*" bivalent (see, below), has a heteromorphic bivalent formed by neo-X and neo-Y chromosomes (neo-XY), similar to that shown by KIAUTA (1983) in specimens from Kyoto and Osaka.

The karyomorph is identical to that occurring in all the specimens from Yakushima

island and from localities North of it (HIGASHI & KAYANO, 1993). The origin of the neo-XY is explicable in terms of a change from XO. A translocation between the X and an "*m*" chromosome, followed by loss of a part of the product, has given a neo-X chromosome. The unpaired "*m*" has remained as a neo-Y (KIAUTA 1983; HIGASHI & KAYANO, 1993).

On the other hand, specimens from the Republic of Korea (Cheju island, Kyongsan, Yondo-ku and Kangnung), and those from Thailand (Ban Bo Luang), showed in primary spermatocytes twelve bivalents and a univalent (n = 13) (Fig.1 D-F). The univalent represents the X and the smallest bivalent, the "*m*" chromosome. The sex de-

Fig. 1. Primary spermatocyte chromosomes at diakinesis or metaphase of *C. servilia* from different localities: (A-C) n = 12 (*C. s. mariannae*) from Iki island (A), Tsushima island (B) and Fukuejima island in Gotoretto Archipelago (C), Japan; — (D-F) n = 13 (*C. s. servilia*) from Chejudo island (D) and Yongdok (E), both Korea, and from Ban Bo Luang, Thailand (F).

termining system is XO. This karyomorph is also found in all specimens from Amamioshima island and from localities South of it (HIGASHI & KAYANO, 1993). It is identic to that reported by KIAUTA (1983) in specimens from continental Asia, China, the Philippines and Taiwan.

From the present and earlier investigations (KIAUTA, 1983; HIGASHI & KAYANO, 1993), it is unequivocal that *C. servilia mariannae* is endemic to Japan; in the South its distribution range reaches Yakushima island, where the western border is represented by Tsushima island and the Gotoretto Archipelago. *C. s. servilia* occurs South of Amamioshima island. The eastern limit of its distribution is Cheju island and mainland of the Republic of Korea (Fig. 2 A, B).

It would be interesting to ascertain the subspecific identity of the populations in the Tokararetto Archipelago, connecting the Amamioshima and Yakushima islands. One of the present authors (K.H.), tried to collect *C. servilia* on the islands of the
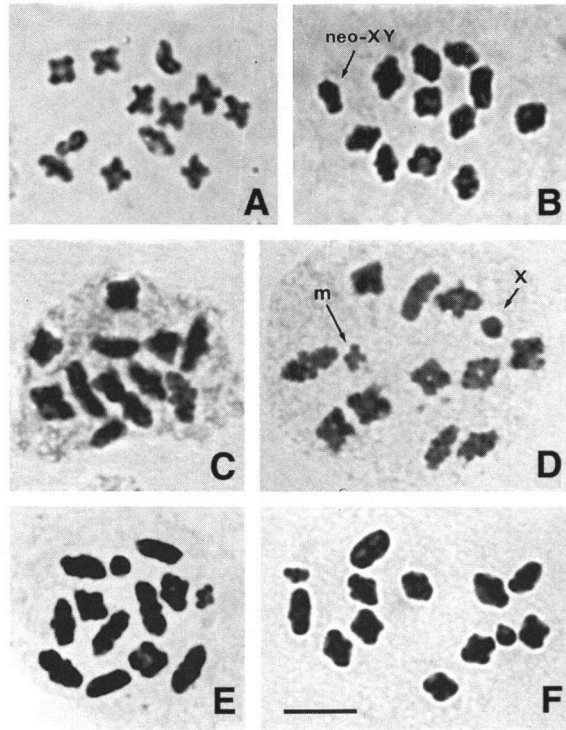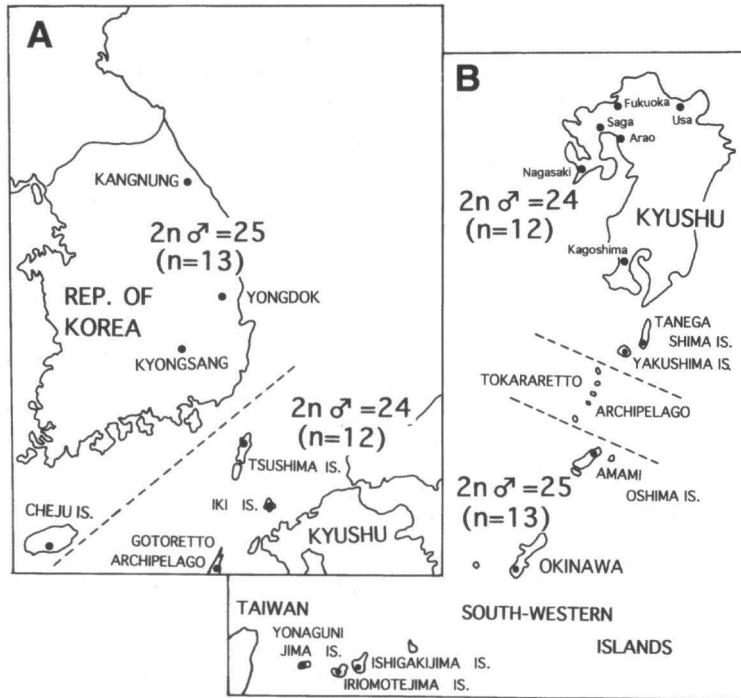
Fig. 2. Distribution of *Crocothemis servilia* karyomorphs: (A) Korea and the islands North of Kyushu, Japan; — (B) Kyushu and the Japanese southwestern islands. — [Dots indicate collecting sites]

Tokararetto Archipelago, i.e. on Kuchinoshima and Takarajima, in mid July 1998, and on Kuchinoshima, Nakanoshima, Akusekijima and Takarajima, in mid July 1999. Unfortunately, the species was not found on any of these.

It is conceivable that *C. s. servilia* individuals could migrate into the area of *C. s. mariannae*, and vice versa. The mechanism to maintain a distinct karyomorph in each area of distribution is discussed below.

We must first consider the possibility of hybridization between the two subspecies. Here, the karyomorph of *C. s. mariannae* is denoted by $2n♀ = 24$ (22+neo-X+neo--X) and $2n♂ = 24$ (22+neo-X+neo-Y) and that of *C. s. servilia*, $2n♀ = 26$ (22+m+m+X+X) and $2n♂ = 25$ (22+m+m+X). In the case of hybridization between *C. s. servilia* (♀) and *C. s. mariannae* (♂), F1's are $2n♀ = 25$ (22+m+X+neo-X) and $2n♂ = 25$ (22+m+neo-Y+X) which is substantially 22+m+m+X and equivalent to the male of *C. s. servilia* (Tab. I, A).

Hybridization between *C. s. mariannae* (♀) and *C. s. servilia* (♂) will produce F1's consisting of $2n♀ = 25$ (22+m+X+neo-X) and $2n♂ = 24$ (22+m+neo-X) which is substantially 22+neo-Y+neo-X and equivalent to the male of *C. s. mariannae*

(Tab. I, B).

The F1 females, 2n ♀ = 25 (22+m+X+neo-X) are partially sterile, because due to random segregation of the m chromosome in meiosis, one half of the eggs are not functional. The F1 females will give four kinds of gametes: (1) n = 12 (11+neo-X), (2) n = 13 (11+m+X), (3) n = 13 (11+m+neo-X) (excess of "m"), and (4) n = 12 (11+X) (shortness of "m"). Among these, types (1) and (2) are genetically balanced (functional in fertilization), but types (3) and (4) are genetically unbalanced (not functional).

Second we consider how foreign chromosomal elements behave after the hybrid females repeatedly back-cross to member(s) of the surrounding "native" population. As mentioned above, the F1 females are partially sterile, irrespective of direction of crosses between the two subspecies. In the case of the surrounding population being *C. s. mariannae*, females in the offspring are of two kinds, one half is *C. s. mariannae* and another half, hybrid type (Tab. II). In the males of back-crossed offspring, one half is *C. s. mariannae* and another half, *C. s. servilia*. In each generation of the offspring after immigration, the theoretical rate of *C. s. servilia* males and hybrid type females decrease to half of the previous generation.

In cases of the hybrids being surrounded by members of *C. s. servilia* (immigration of *C. s. mariannae* into a population of *C. s. servila*), back-crosses will give offspring in which one half of the females is hybrid type and the other half is *C. s. servilia* (Tab. II). In males of the back-crossed offspring, one half is *C. s. servilia* and the other is *C. s. mariannae*. Theoretically, the rate of *C. s. mariannae* males and hybrid type females decrease to half of the previous generation.

Therefore, as long as the immigrants are rare in the population, it is likely that the foreign karyomorph introduced by the immigrants will disappear from the population in a few generations. In fact, exchange of individuals between the populations of different karyomorphs is very restricted because the two karyomorphs are distributed in different areas, separated by the sea over a long distance.

Table I

Presumptive chromosome constitutions of hybrids (F1) resulting from crosses between two subspecies of *C. servilia*; in F1 males "m+neo-Y" is equivalent to "m+m" and unpaired "m" is substantially "neo-Y"

(A) *C. s. mariannae* (♂) × *C. s. servilia* (♀)	
Sperm	Egg n = 13 (11+m+X)
n = 12 (11+neo-X)	(2n♀ = 25) 22+m+X+neo-X hybrid type
n = 12 (11+neo-Y)	(2n♂ = 25) 22+m+neo-Y+X *servilia* type

(B) *C. s. servilia* (♂) × *C. s. mariannae* (♀)	
Sperm	Egg n = 12 (11+neo-X)
n = 13 (11+m+X)	(2n♀ = 25) 22+m+X+neo-X hybrid type
n = 12 (11+m)	(2n♂ = 24) 22+m+neo-X *mariannae* type

Table II

Presumptive chromosome constitutions expected in offspring resulting from back-crosses between females of F1 hybrid from *C. s. servilia* (♂) × *C. s. mariannae* (♀) or vice versa and "native" males; (a-1, a-2): sperm from *C. s. mariannae*; (b-1, b-2): sperm from *C. s. servilia*: in males "*m*+neo-Y" is equivalent to "*m*+*m*" and unpaired "*m*" is substantially "neo-Y"

Sperm	Egg n = 12 (11+neo-X) ·	Egg n = 13 (11+*m*+X)
(a-1) n = 12 (11+neo-X)	(2n ♀ = 24) 22+neo-X+neo-X *mariannae* type	(2n ♀ = 25) 22+*m*+X+neo-X hybrid type
(a-2) n = 12 (11+neo-Y)	(2n ♂ = 24) 22+neo-X+neo-Y *mariannae* type	(2n ♂ = 25) 22+*m*+neo-Y+X *servilia* type
(b-1) n = 13 (11+*m*+X)	(2n ♀ = 25) 22+*m*+X+neo-X hybrid type	(2n ♀ = 26) 22+*m*+*m*+X+X *servilia* type
(b-2) n = 12 (11+*m*)	(2n ♂ = 24) 22+*m*+neo-X *mariannae* type	(2n ♂ = 25) 22+*m*+*m*+X *servilia* type

Geographic isolation is a primary cause of maintaining the distinctness of karyomorphs of the two subspecies.

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