HYBRIDIZATION BETWEEN *ANOPHELES SINENSIS* AND *ANOPHELES SINEROIDES*[1]

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ABSTRACT. Three strains of *Anopheles sinensis* and *An. sineroides* respectively collected in Japan north to south were used for the present hybridization experiments to determine genetic affinities among strains of *sineroides* and between *sinensis* and *sineroides*. Comparing the F_1 hybrids and those of their parent species for the control, there exists no difference in the number of ova laid and their percent hatches. The F_1 hybrids' salivary chromosomes which showed complete asynapsis verify the observation that the two entities differ chromosomally by band differences formed in their paternal entities at the telomer in region of the x and the pericentric area of 3R of their chromosomes. The low viability of the F_1 hybrids with high larval and pupal mortalities, and the fact that only a few F_1 hybrids were obtained suggest there exists reproductive isolation between both entities. The data presented suggest that *An. sinensis* and *An. sineroides* are two separate species.

INTRODUCTION. *Anopheles sinensis* Wiedemann, 1828 occurs in East Asia from north to south and west to Assam. Harrison (1973) applied the name "*sinensis*" to a variable common Oriental member of the *hyrcanus* species group (Reid 1953, 1968). On the other hand the distribution of *Anopheles sineroides* Yamada, 1924 is more restricted. It occurs from Hokkaido to Kyushu in Japan (Kamimura 1968; Sasa 1971), in China (Feng 1938; Reid 1953; Xu and Feng 1975) and in Korea (Yamada 1936). Because of a great deal of similarity in morphological characters and sometimes in ecological characters, genetic affinities between the two entities must also be confirmed. The present authors attempted to conduct a hybridization experiment between the two entities.

MATERIALS AND METHODS. The strains of *sinensis* and *sineroides* used in the present experiment were collected from Kanoya, Karuizawa and Engaru, and from Karuizawa, Engaru and Mombetsu respectively.

Collection, maintenance and rearing of the mosquitoes were performed by the methods similar to those described by Oguma and Kanda (1976); for identification of the two entities, wing spots of the adults collected and decks of the eggs were classified according to Ohtsuru and Ohmori (1960). Salivary chromosome preparations were made as reported by French et al. (1962) and modified by Kanda (1971). All crosses shown in Table 1 were made by induced copulation following a method slightly altered from that originally described by Baker and Kitzmiller (1961).

RESULTS. The results of intraspecific crosses of *sinensis* and *sineroides* for control as well as the interspecific crosses are shown in Table 1. While the numbers of eggs laid between interspecific crosses and their controls are quite similar, the slight decrease in percent hatch from the F_1 hybrid eggs may be seen; and most of those hybrids died in the larval stage. Only 6 adults were obtained from a total of 495 hatched eggs of F_1 hybrids between *sinensis* and *sineroides*. This small number of F_1 hybrids precluded any possibility of testing male fertility.

The salivary chromosomes of the F_1 hybrids in intraspecific crosses of the Karuizawa strain and the Mombetsu

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Table 1. Results of crosses.

	No. of ova batches	Total no. of ova	Percent hatch
Controls			
sinensis	3	675	53.9
sineroides	3	554	75.6
Crosses			
KIZ-S x KIZ-SRD	3	599	49.2
ENG-S x KIZ-SRD	7	998	20.0

KIZ-S: Karuizawa strain of *sinensis*, ENG-S: Engaru strain of *sinensis*.
KIZ-SRD: Karuizawa strain of *sineroides*.

strain of *sineroides* showed complete synapsis. The males and the females of the F_1 hybrids were fertile. These strains, therefore, are recognized to be genetically conspecific. Figure 1 shows complete asynapsis and the characters of the parental chromosomes of the salivary gland of the F_1 hybrids between both the Karuizawa strains of *sinensis* and *sineroides*. In the comparisons of the banding patterns of the salivary chromosomes between *sinensis* and *sineroides*, 2 differences found between both parental species and also in the F_1 hybrids are as follows: one is in the banding patterns at the distal end of the X and the other is in the bands at the centromeric area in 3R. These 2 differences on the banding patterns were consistently observed. The chromosomes from the F_1 hybrids between the Engaru strain of *sinensis* and the Karuizawa strain of *sineroides* also showed a similar structure as in the sympatric crosses between *sinensis* and *sineroides* of the Karuizawa strains. All of these cytogenetic data support the conclusion that *sinensis* and *sineroides* are separate species.

DISCUSSION. Because of the variation in the external morphological characters of *sinensis*, prior to 1953 the species name of *sinensis* was treated as a subspecies of *Anopheles hyrcanus* (Pallas) 1771. Reid (1953) treated *sinensis* as a species which is included in the *hyrcanus* species group. Ohtsuru and Ohmori (1960) also considered it a

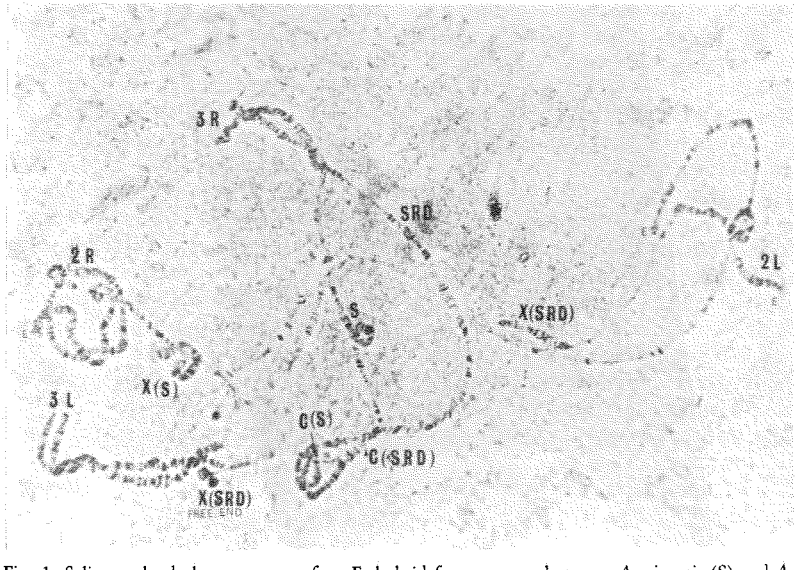

Fig. 1. Salivary gland chromosomes of an F_1 hybrid from crosses between *A. sinensis* (S) and *A. sineroides* (SRD).

species. Harrison (1973) established the true identity of *sinensis* by the designation and description of a lectotype, because the name *"sinensis"* could not be assigned with certainty to a particular species.

The existence of reproductive isolation between *sinensis* and *sineroides* is suggested by the present results: 1) viability disturbance in the F_1 hybrids with extreme reduction in the number of the F_1 hybrid males and females, and 2) complete asynapsis of the salivary chromosomes of the F_1 hybrids between the two entities. While the cytogenetic data of *sineroides* suggest the existence of apparent genetic divergence from *sinensis*, the banding patterns of the X chromosome of the salivary gland of *sineroides* are similar to those of *Anopheles koreicus* (Oguma and Kanda 1970) rather than of *sinensis*. This fact furnishes impetus for a study of further genetic relations between these 3 or more entities. On the other hand the affinity between *sinensis* and another member of the *hyrcanus* species group, *lesteri*, which showed a great deal of chromosomal homology in most of the corresponding arms is published in a separate paper (Kanda and Oguma, *Mosquito News,* this number).

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References Cited

Baker, R. H. and J. B. Kitzmiller. 1961. Induced copulation and chromosomal heterozygosity in *Anopheles*. Amer. Zool. 1:455.

French, W. L., R. H. Baker and J. B. Kitzmiller. 1962. Preparation of mosquito chromosomes. Mosquito News 22:377–383.

Feng, L. C. 1938. A critical review of literature regarding the records of mosquitoes in China. Part I. Subfamily Culicinae, Tribe Anophelini. Peking Nat. His. Bull. 12:169–181, 318–385.

Harrison, B. A. 1972. A. new interpretation of affinities within the *Anopheles hyrcanus* complex of Southeast Asia. Mosquito Systematics 4:73–83.

Harrison, B. A. 1973. A lectotype designation and description for *Anopheles (An.) sinensis* Wiedemann, 1828, with a discussion of the classification and vector status of this and some other Oriental *Anopheles*. Mosquito Systematics 5:1–13.

Kanda, T. 1971. Chromosomes of mosquitoes. Recent advances in medical entomology. 1:105–135. Keigaku Shuppansha, Tokyo.

Kamimura, K. 1968. Distribution and ecology of mosquitoes of medical importance in Japan. Japn. J. Sanit. Zool. 19:15–34.

Oguma, Y. and Kanda, T. 1970. The salivary gland chromosomes of *Anopheles koreicus*. Japan. J. Sanit. Zool. 21(2):114.

Oguma, Y. and T. Kanda. 1977. Laboratory colonization of *Anopheles sinensis*. Japan J. Sanit. Zool. (in press).

Ohtsuru, M. 1953. A new race of *Anopheles hyrcanus* found in Japan. Hukuoka Igaku Zashi 40:139–148.

Ohtsuru, M. and Ohmori, Y. 1960. Malaria studies in Japan after World War II. The research for *Anopheles sinensis* sibling species group. Japan. Exp. Med. 30:33–65.

Reid, J. A. 1953. The *Anopheles hyrcanus* group in Southeast Asia. Bull. Entomol. Res. 44:5–76.

Reid, J. A. 1968. Anopheline mosquitoes of Malaya and Borneo. Studies Inst. Med. Res. Malaya No. 31, 520.

Sasa, M. 1971. An index and revisional discussion on the taxonomy of mosquitoes in Japan. Recent advances in medical entomology No. 1:1–47. Keigaku Shuppansha, Tokyo.

Xu, J. J. and Feng, L. C. 1975. Studies on the *Anopheles hyrcanus* group of mosquitoes in China. Acta Entom. Sin. 18:77–98.

Yamada, M. 1936. Four kinds of anopheline mosquitoes in Chosen. Keijo J. Med. 7(2):191–210.