CHROMOSOMAL POLYMORPHISM AND SALIVARY GLAND CHROMOSOMES OF HYBRIDS BETWEEN STRAINS OF *ANOPHELES SINENSIS* (DIPTERA: CULICIDAE)¹⁾

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Anopheles sinensis (Wiedemann 1828) is a member of the *A. hyrcanus* species group and the most wide-spread species in this species group. The distribution of the *A. sinensis* ranges from Japan and Korea in north, southwards through China to the Malay Peninsula. In southeast Asia, Assam is the weatern limit; it is absent from the rest of India (Reid 1968).

It is the medically important vector of a filaria, *Brugia malayi* (Filariae), in China and Korea (Feng 1931; Kanda *et al.* 1975) and is the supposed vector of malaria in southeast Asia. The speciation and evolution within *A. sinensis* as well as *A. hyrcanus* species group is of interest to geneticists and taxonomists and genetical analysis of these problems is now required.

In general, a considerable amount of genetic variability concealed in natural populations of outbreeding diploid organisms. Some local populations of *A. sinensis*, the Tomakomai and the Engaru strains, are different than other *A. sinensis* strains with respect to the external morphology and the frequencies of the clasper movement in the male mosquitoes during induced copulation (Kanda and Oguma 1976). About 23% of the Tomakomai and the Engaru strains of *A. sinensis* had H p (humeral pale spots of wings), but that character could not be found in other *A. sinensis* strains. The mean frequencies of the clasper movements in the male adults of the Tomakomai and the Engaru strains of *A. sinensis* were 14.3 and 14.6, respectively, per one copulation induced and that of the original *A. sinensis* strain was 8.1. The difference in the frequencies of both was significant at the 1% level. Chromosomal polymorphism and the salivary gland chromosomes of hybrids in the strains have been studied in the present paper to see if the cytogenetic data might be correlated with the morphological observations and possible genetic divergence.

MATERIALS AND METHODS

For the study of chromosomal polymorphism in the strains of *A. sinensis* the materials were obtained at 11 localities, *i.e.* Koniya, Kanoya, Arai, Tokushima, Oka-

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Fig. 1. Collecting localities of *Anopheles sinensis*.

yama, Yodoe, Ama, Fukumitsu, Karuizawa, Natori and Engaru, as given in Fig. 1. The methods of mosquito collection and rearing in the laboratory were the same as described in Oguma and Kanda (1976). About 50 females caught at each locality were allowed to lay eggs; these were reared to the fourth instar, then used for preparation of the salivary gland chromosomes. The techniques for preparation of the chromosome slides were similar to those described by French *et al.* (1962) and Kanda (1971). The number of the chromosomes observed was 60 for each of the arms except in the Koniya strain, in which it was 260; for a comparison of the chromosomal polymorphism, the salivary gland chromosomes of hybrids were made by induced copulation between any two of the Kanoya, Yakumo and Engaru strains.

RESULTS

Although two inversions, In2RA(12D-14B) and In3RA(28A-31B), had been found in the salivary gland chromosomes of this species (Kanda, unpublished), another simple

Table 1. Chromosomal variability (%) in *Anopheles sinensis* at 11 localities

Locality	No. of chromosomes observed	Chromosome 2R		Chromosome 3R	
		ST*	In2RB	ST	In3RA
Engaru	60	1.65	98.35	95.00	5.00
Natori	60	100	0	100	0
Karuizawa	60	100	0	100	0
Fukumitsu	60	100	0	98.35	1.65
Ama	60	100	0	95.0	5.00
Yodoe	60	100	0	100	0
Okayama	60	100	0	100	0
Tokushima	60	100	0	100	0
Arao	60	100	0	100	0
Kanoya	60	100	0	98.35	1.65
Koniya	260	100	0	100	0

* Standard arrangement

Fig. 2. Salivary chromosome complement of an F_1 between the Kanoya strain and the Yakumo strain of *Anopheles sinensis*.

Fig. 3. Salivary chromosome complement of an F_1 between the Yakumo strain and the Kanoya strain of *Anopheles sinensis*.

Fig. 4. Salivary chromosome of the Engaru strain of *Anopheles sinensis*.

Fig. 5. Salivary chromosome complement of an F_1 between the Engaru strain and Yakumo strain of *Anopheles sinensis*; Incomplete synapsis along the whole lengths of the chromosome arms.

inversion, In2RB, was observed in the Engaru population, located at 9A-11D on the right arm of the second chromosome. All the other regions of the chromosomal arms in the strains examined seemed to be the same as in the standard arrangement, and after all, the above mentioned three inversions have been found as polymorphisms of the salivary gland chromosomes in this species as summarized in Table 1. Inversion In2RA, which Kanda had found, could not be seen in any of the populations examined. Inversion In2RB was found only in the Engaru population and not in any other populations, whereas inversion In3RA was seen in the Engaru, Fukumitsu, Ama and Kanoya populations, although with very low frequencies at all of these localities.

Figs. 2 and 3 show the chromosome complements in the salivary glands of the hybrids between the female of the Kanoya and the male of the Yakumo strain, and of its reciprocal cross; in both the figures they are completely synaptic, although there is a small asynaptic region near the centromere in 3R. This small asynaptic region can usually be observed in *A. sinensis* and also even in the same strain, therefore this asynapsis is not due to hybridization between the two strains. The salivary gland chromosomes of the Engaru strain, which are shown in Fig. 4, show homologous pairing in the region of In2RB.

The hybrid chromosomes obtained from crosses between females of the Engaru and males of either the Yakumo or the Kanoya strains are presented in Figs. 5 and

Fig. 6. Salivary chromosome complement of an F_1 between the Engaru strain and Kanoya strain of *Anopheles sinensis*; Incomplete synapsis along the whole lengths of the chromosome arms.

6; in these figures incomplete synapsis is observed along the whole length of the chromosomal arms. In2RB is seen on the right arm of the second chromosome, while the X-chromosomes are not paired in Fig. 5, but are loosely paired in Fig. 6. When the Tomakomai strain was crossed with the Kanoya strain, the hybrid chromosomes showed complete synapsis in some crosses and incomplete synapsis in other crosses. Both complete and incomplete synapsis in this cross occurred with an almost equal frequency.

DISCUSSION

The investigation of inversion polymorphism has been useful for the study of evolution of species particularly in some families of Diptera. The most advanced and detailed investigations of this sort have been carried out in *Drosophila*. Anopheline mosquitoes are also favorable for the study of inversion polymorphism, for the salivary glands are good materials for the preparation of polytene chromosomes. The salivary gland chromosomes in *A. maculipennis* complex and in *A. gambiae* complex have been studied by Kitzmiller *et al.* (1967) and Davidson *et al.* (1967), respectively, who des-

cribed many chromosomal inversions in these species, although with very little information on the quantitative aspects of the chromosomal polymorphism, whereas Coluzzi (1972) made a quantitative study of inversion polymorphism in 4 strains of *A. stephensi*.

Although a satisfactory quantity of materials for this study could not be acquired because of difficulties of preparation of the salivary gland chromosomes of *A. sinensis*, these data still can be useful for study of this species. Inversion In2RB exists in the Engaru population with a high frequency but was never found in any other populations thus far studied; this fact suggests that there is genetic divergence between the Engaru strain and strains of the original *A. sinensis*. Inversion In3RA is probably a common inversion, because it is found in populations from northern to southern parts of Japan with a low frequency. Inversion In2RA was found by Kanda (unpublished) from the Konosu population in Saitama Prefecture, near Tokyo and might exist in the populations with lower frequency than the In3RA. The salivary gland chromosomes of the F_1 between the Kanoya and the Yakumo strains showed complete synapsis, suggesting that both the populations belong to the same species population. Incomplete synapsis of the salivary gland chromosomes was found in the F_1 between the Engaru and either the Kanoya or the Yakumo strain; the homologous chromosomes did not become completely parallel, and were loosely synaptic and twisted with each other in some parts.

According to Davidson *et al.* (1967), in the salivary gland chromosomes of the F_1 of *A. gambiae* A and B, the X-chromosomes and 7-11 of 2R are completely asynaptic; translocations are found between 2L and 3R; 3L is completely synaptic along this arm. In the *A. maculipennis* complex, the salivary gland chromosomes of the F_1 of *A. freeborni*×*aztecus*, *freeborni*×*earlei*, *freeborni*×*occidentalis* and *earlei*×*occidentalis* have been observed by Kitzmiller *et al.* (1967) to be completely asynaptic. The above species complex studied by Kitzmiller *et al.* can be identified by the banding patterns alone, on the other hand *A. sinensis* can not be distinguished from *A. lesteri* by the banding patterns of the salivary gland chromosomes, although the F_1 of both the species show completely asynaptic chromosomes (Kanda and Oguma, unpublished). Therefore the species which have homosequential banding patterns do not always show synapsis in their hybrid chromosomes. Based on the degree of synapsis it appears that there is more genetic divergence between the Engaru and Kanoya strains rather than between taxa A and B of *A. gambiae*, but less divergence than among *A. maculipennis* complex or between the *A. sinensis* s.l. and *A. lesteri*.

In the case of *Drosophila*, *Drosophila bifasciata* inhabits extensive areas in Europe and Asia including Japan, most commonly in cool and mountainous districts, while *D. imaii* has been collected only in Hokkaido. The salivary gland chromosomes in the F_1 of these species were observed to be incompletely synaptic in the second chromosome and the right arm of IV, but showed complete synapsis in the other arms (Moriwaki *et al.* 1967). They indicated that *D. imaii* was a new sibling species of *D. bifasciata*. By the same logic the Engaru strain can be considered to be a sibling species of the former *A. sinensis*. The salivary gland chromosomes of the F_1 of the Tomakomai and Kanoya strains showed complete synapsis in some crosses and complete asynapsis in other crosses; this probably is because *A. sinensis* of the Engaru type and the original *A. sinensis* were mixed in the Tomakomai population. Further study is required to

obtain the answer to this question.

SUMMARY

A new kind of inversion, named In2RB, has been found in the Engaru population of *Anopheles sinensis*. This is a simple inversion located at 9A-11D on the right arm of the second chromosome. Inversion polymorphism was investigated on In2RA, In2RB and In3RA in material from 11 localities. In2RA was not observed at all the localities and In2RB was found only in the Engaru population. In3RA was observed in four strains with low frequency. The hybrid chromosomes obtained from crosses between the Engaru strain and either the Kanoya or the Yakumo strains showed incomplete synapsis along the whole lengths of the chromosome arms.

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