

HYBRIDIZATION BETWEEN *ANOPHELES SINENSIS* AND *ANOPHELES LESTERI*¹

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ABSTRACT. Hybridization experiments among 5 strains of *lesteri* as interstrain crosses and between 4 strains of *sinensis* and 2 strains of *lesteri* as interspecific crosses were performed. All the strains used originated from different localities in Japan. Results of interstrain crosses of *lesteri* indicated that reproductive isolation does not exist among the strains of this species. The fact that *sinensis* and *lesteri* co-exist in Japan from north to south and retain their identities is by itself evi-

dence that they are true biological species. Reproductive isolation due to hybrid inviability and complete chromosomal asynapsis of the F₁ hybrids between *sinensis* and *lesteri* confirm this, while synaptic x chromosomes were exceptionally found in a few cases of the F₁ hybrids. Many similarities in banding patterns of the salivary chromosomes observed in the F₁ hybrids and their parental species indicate a close relationship.

INTRODUCTION. *Anopheles lesteri* (Baisas and Hu 1936) occurs in East Asia from Hokkaido to Malaysia, south to the Indonesian territory, north into continental China and west into the Pacific Islands (Reid 1953; Ohtsuru 1949; Miyazaki 1951; Kamimura 1968; Sasa 1971; Feng 1938; Xu and Feng 1975), breeding in low density. On the other hand *An. sinensis* can be found commonly in East Asia, including Japan. Morphological similarities between *sinensis* and *lesteri* make identification difficult. Therefore *lesteri* was sometimes treated as *sinensis* or insufficient characters were used for the identification in the areas where *lesteri* occurs (Harrison 1973). While morphological differences were definitely pointed out by Reid (1953, 1968) and Harrison (1973), the present authors attempted hybridization experiments to study genetic relationships.

MATERIALS AND METHODS. Specimens of *sinensis* used in the study were collected at Kanoya, Okayama, Karuizawa and Engaru, and of *lesteri* at Okayama, Yomogita, Yakumo,

Iwamizawa and Engaru. Those localities are shown in Figure 1. The Yakumo strain of *lesteri* has been maintained by means of induced copulation in the laboratory and the other strains were maintained for a few generations. The methods for maintaining all of those colonies were similar to those reported by Oguma and Kanda (1977).

The identification of adult anopheline mosquitoes and their progeny (eggs, larvae, pupae and adults) were performed by checking their morphological characters among the members of the *byrcanus* species group by criteria established by Reid (1968), Ohtsuru and Ohmori (1960) and Harrison (1973).

The method used for the preparation of salivary gland chromosomes was similar to the method described by French et al. (1962) and Kanda (1971). All the crosses shown in Tables 1 and 2 were made by induced copulation, being similar to methods reported by Baker and Kitzmiller (1961) and Kanda and Oguma, *Mosquito News*, this number.

RESULTS AND DISCUSSION. The results from the crosses among interstrains of *lesteri* are shown in Table 1. There exist no appreciable differences

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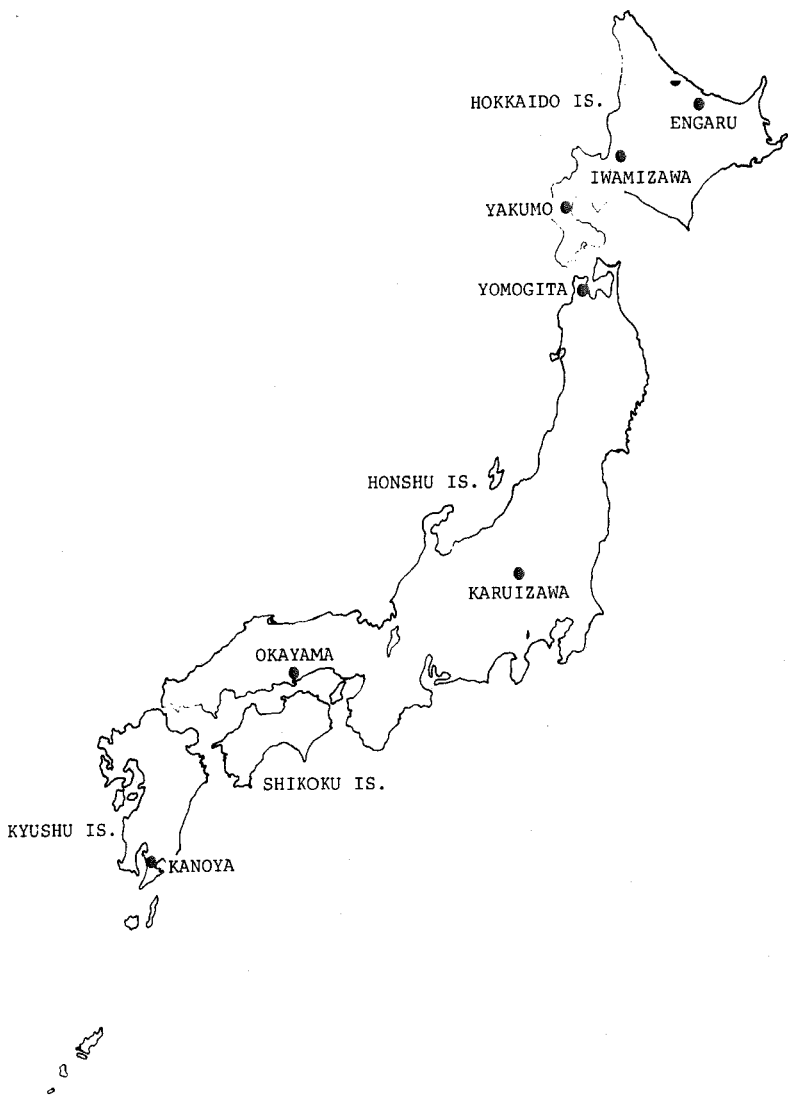


Fig. 1. A Japanese map indicating the localities of anopheline mosquitoes collected.

Table 1. Results from crosses among interstrains and their backcrosses and $F_1 \times F_1$ crosses in *lesteri*.

	No. batches	No. ova in amount	Percent hatch	Fertility
Interstrain				
OKY x YGT	5	1248	10.9	F
OKY x YKM	4	1129	13.2	F
YKM x OKY	7	948	5.7	F
Backcrosses				
(OKY x YGT) F_1 x OKY	6	1969	22.4	F
OKY x (OKY x YGT) F_1	4	997	11.6	F
$F_1 \times F_1$ crosses				
(OKY x YKM) F_1 x (OKY x YKM) F_1	4	1016	11.8	F
(YKM x OKY) F_1 x (YKM x OKY) F_1	5	1063	6.1	F
(IMZ x YKM) F_1 x (IMZ x YKM) F_1	6	1040	1.8	F
(YKM x ENG) F_1 x (YKM x ENG) F_1	5	F

ENG: Engaru, IMZ: Iwamizawa, OKY: Okayama, YGT: Yomogita, YKM: Yakumo.

in oviposition and hatchability in the comparison with interstrain and backcrosses, although the numbers of ova laid and percent hatches were lower than those of natural matings of the parental species (Oguma and Kanda 1976). Fertilities of the F_1 hybrids between the strains of *lesteri*, backcrosses

and $F_1 \times F_1$ of *lesteri* were investigated. The data show that the F_1 hybrid males and females were fertile in all crosses. The salivary gland chromosomes of the F_1 hybrids did not show any asynaptic area nor heterozygous banding patterns (Figure 2).

The results of crossing experiments



Fig. 2. Entirely synaptic salivary gland chromosomes from an F_1 hybrid of interstrain cross (Yakumo x Okayama) of *lesteri*.



Fig. 3. Entirely asynaptic salivary gland chromosomes from an F₁ hybrid of (Kanoya strain of *sinensis* x Okayama strain of *lesteri*).

Fig. 4. Salivary gland chromosomes with synaptic x instead of asynaptic autosomes from an F₁ hybrid of (Okayama strain of *lesteri* x Kanoya strain of *sinensis*).

Table 2. Results from crosses of parental species and interspecies *sinensis* and *lesteri*.

Crosses	No. batches	No. ova in amount	Percent hatch	Fertility
Paternal species				
<i>sinensis</i>	12	2946	47.5	F
<i>lesteri</i>	3	1130	27.6	F
Inter species				
<i>lesteri</i> x <i>sinensis</i>	11	2959	10.5	unable to test
<i>sinensis</i> x <i>lesteri</i>	10	2007	16.0	unable to test

performed between *sinensis* and *lesteri* are shown in Table 2. In the interspecific crosses percent hatches were relatively lower in the F₁ hybrids than that of the parental entities. The F₁ hybrids also showed high mortality in the larval and pupal stages. Almost all individuals of these crosses could not emerge and consequently the fertility of the reciprocal F₁ hybrids could not be assessed.

The salivary gland chromosomes of the F₁ hybrids were observed in crosses between the Kanoya strain of *sinensis* and the Okayama strain of *lesteri* (Figure 3), and between *lesteri* and *sinensis*, both collected at Okayama. These F₁ hybrids were characterized by complete chromosomal asynapsis except for a few exceptional individuals having some synaptic x chromosomes (Figure 4). The banding patterns of both haploid chromosomes from the paternal species in the F₁ hybrids were similar, although minor differences may exist.

Certain morphological characters used by Ohtsuru and Ohmori (1960) were checked on adults collected as well as eggs, larvae, pupae and adults of the progeny for the identification of *lesteri* and *sinensis*. Other reliable characters described by Harrison (1973) were reconfirmed on each strain. Harrison (1973) noted that while some of the characters seemed to be identical in *lesteri*, they were highly variable in *sinensis*, therefore *lesteri* was regarded as one of the cryptic species hidden under the

name of "*sinensis*" in surveys such as in China. The large number of external morphological similarities, as mentioned above, and also the similarity in banding patterns of the salivary chromosomes between the two suggest that they are separate but very closely related species.

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INCREASED ABRASION AND WASH RESISTANCE OF REPELLENTS WITH ADDITION OF POLYMERS

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ABSTRACT. Repellents applied to skin are lost with sweating, water exposure, or on abrasion with clothes or other body parts. Attempts were made to increase water washability and abrasion resistance of repellents by formulating with a commercially available polymer formulation. Mixing with the polymers improved water washability several fold. Formulation with polymers was most effective with triethylene glycol monohexyl

ether and least with dimethyl phthalate. Deet in formulation was improved in water washability 11 times. Formulation with polymers improved abrasion resistance. Deet with polymers resisted abrasion significantly. In four of five tests deet with polymer remained effective for approximately 24 hr. The polymers used appeared innocuous but feel sticky when freshly applied. Improvement is required for cosmetic acceptability.

INTRODUCTION

Mosquito repellents protect best under dry conditions. With heavy sweating or water exposure they fail in minutes. Abrasion with clothing and friction against body parts reduce effectiveness considerably. Under rigorous field conditions (for professional sportsmen, foresters, and military personnel), available repellent formulations often need frequent applications. There is a need to improve abrasion and water-wash resistance of repellents so that a single application may last several hours under demanding field conditions.

We describe the effect of improved abrasion and wash resistance of mosquito repellents by formulating them with a commercially available polymer (Areoplast® dressing, Parke-

Davis & Co.) containing co-polymers of hydro-vinyl chloride-acetate and sebacic acid, 8.2%; modified maleic rosin ester, 2.7%; and glycolate plasticizer, 0.6% (total solids 11.5% by weight) in ethyl acetate-acetone solvent.

MATERIALS AND METHODS

The polymer formulation (Aero-plast®) was emptied in a beaker and mixed with an appropriate amount of repellent. All quantities reported in the text pertain to the weight of the solids in the polymer formulations. The solvent was not removed before application. A 5 x 12.5 cm area was marked on the ventral surface of a human forearm and a measured amount of repellent or its formulation with the polymer was applied with a fine pipette, covering the area