Anopheles engarensis, a New Species Related to *sinensis* from Hokkaido Island, Japan

Tozo Kanda and Yuzuru Oguma
Department of Medical Zoology
St. Marianna University School of Medicine
2095, Sugao, Takatu, Kawasaki, 213, Japan

ABSTRACT. A new species, *Anopheles engarensis* is named and described. This species has been separated from Japanese populations of *Anopheles sinensis* on the basis of hybridization studies, chromosomal studies and certain variable morphological characters.

INTRODUCTION

Anopheles (A.) sinensis is a common member of the *hyrcanus* species group (Reid, 1953, 1968; Harrison, 1973). The present authors (Kanda and Oguma, 1976) have reported on a subgroup of this species which was observed to have different behavior during induced copulation. This subgroup with different behavior has been subsequently studied genetically (to be reported in a separate paper) and cytogenetically (Oguma, 1976). In reciprocal crosses between typical *sinensis* from other parts of Japan and the Hokkaido strain from Engaru, F_1 males were consistently sterile but females were partially fertile in backcrosses. Full fertility in both males and females was recovered in the BC_2 generation.

No spermatogenesis was observed in the testes of F_1 males. The X chromosomes and the autosomes of F_1 hybrid larvae are partially asynaptic. The Engaru strain has a consistent fixed paracentric inversion in the right arm of chromosome 2 (2Rb, Oguma, 1976), not found in other strains of *sinensis*. The Engaru strain of *sinensis* had been given the tentative name, in previous papers, of *Anopheles sinensis* "E", or *Anopheles sinensis*-Engaru. On the basis of the hybridization and sterility data, the fixed inversion in 2R, the unique copulatory behavior and certain morphological characters described below, the Engaru strain of *Anopheles sinensis* is hereby designated a full species, *Anopheles engarensis* Kanda and Oguma. The summarized data of those characters were reported by Kanda et al. (1976).

MATERIALS AND METHODS

Mosquitoes used for morphological observation were the F_1 offspring of about 70 blood fed females of *sinensis* and *engarensis* collected in the field and maintained by the methods previously reported by Oguma and Kanda (1976). Collection sites were as follows: Urawa, approximately 10 Km north of Tokyo; Yungjyu, about 150 Km northeast of Taegu, Kyungpook, South Korea; and Engaru, a town in the northeast part of Hokkaido Island. Each egg batch group was reared separately.

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Two ♂s and two ♀s from each of 50 egg batches were used for the observations. The cytogenetic methods are similar as reported by Oguma (1976). Each adult specimen is matched with its pupal and 4th instar larval skin. Ten eggs from each of 5 batches which were confirmed from siblings to be the species indicated were measured. The details of the methods for measurements of eggs were similar to those reported by Ohtsuru et al. (1951).

Anopheles engarensis New Species

TYPE DATA

Types. One female and one male with their matching pupal and larval skins on slides, and several females and males without skins, were presented to the Brit. Mus. (Nat. Hist.), London. The female with skins is selected to be the HOLOTYPE of *Anopheles engarensis*. It can be recognized by three labels on the pin; the second reads, colony 6, VIII, 73 Engaru Hokkaido Island, Japan, and the third reads No. 13 female. The male allotype is similarly labeled except that the third label reads No. 9 male.

Paratypes: 100 females and 100 males with associated larval and pupal skins. Two males and two females were selected from each of 50 egg batches (1-1, 1-2, 1-3, 1-4; 2-1, 2-2, 2-3, 2-4; 3-1----- etc.). In each group of 4, specimens -1 and -2 are females, -3 and -4 are males. In addition, 21 specimens from each of 50 egg batches have been deposited, together with the paratypes in the collection in the Department of Medical Zoology, St. Marianna University School of Medicine, Kawasaki, Japan.

MORPHOLOGY

Morphological characteristics are variable among different strains of *sinensis*, but certain features show trends which may help to separate *engarensis* from *sinensis*. These are listed below.

FEMALES

sinensis: palps with last two pale bands often broad, sometimes tending to fuse; few to many scattered white or pale gray scales between the pale bands.

Wings with humeral cross vein with scales; costa with few to many scattered pale scales between base and subcostal pale spot, often mainly on the posterior border of the costa; sometimes also a few pale scales on the front edge of the costa between base and subcostal pale spot, often mainly on the posterior border of the costa; sometimes also a few pale scales on the front edge of the costa between base and humeral cross vein. Vein 1 often with many scattered pale scales between the subcostal and preapical pale spots. Subcosta usually with some scattered pale scales before the subcostal pale spot. Extreme base of vein 5 often with a small dark mark or a few dark scales.

engarensis: palps of female with pale bands narrow and well defined, last two not broad or tending to fuse, few or no scattered pale scales between the pale bands.

Wings with humeral cross vein bare. Costa usually without or with very few scattered pale scales between base and subcostal pale spot, though there may be some on the front edge or ventrally between base and humeral cross vein. Vein 1 usually all dark or with only a few pale scales between subcostal and preapical pale spots. Subcosta usually without any obvious scattered pale scales before subcostal pale spot. Extreme base of vein 5 all pale.

PUPAE

Setae IV5 + V5 with more branches in *sinensis* than in *engarensis*. The ratio of width to length of lateral spines on VII is smaller in *sinensis*; most *sinensis* specimens have dark spots on wing bag.

LARVAE

Branches of setae on 115 + V5 are fewer in *sinensis* than in *engarensis*.

OVA

The length and width of the ova are similar in both species. The ratio of width of deck to width of egg was .59 $\pm$ .036 in *sinensis* and .634 $\pm$ .029 in *engarensis*.

The details of these data are shown in tables 1 and 2. The morphological differences between the two entities treated as *sinensis* in the past are difficult to verify in individual specimens because of overlapping, but they may be separated qualitatively as shown in the tables.

DISTRIBUTION AND BIOLOGY OF *ANOPHELES ENGARENSIS*

Detailed data will be published in a separate paper by Oguma and Kanda. The new species was previously found in 1970 at Omu, Monbetsu, Engaru, Obihiro and Furenai; in 1971 at Engaru, Iwamizawa and Tomakomai; in 1972 at Kotambetsu, Engaru, Fukagawa and Tomakomai; in 1973 it was only collected at Engaru and in 1974 it was collected in Wakkanai, Engaru and Kushiro. The distribution of *engarensis* is supposedly in northeastern Hokkaido and is limited more or less up to Kotambetsu and Tomakomai. Larvae usually breed in rice fields and are also found in small creeks and ponds; the species is therefore sympatric with *sinensis, lesteri* and at times with *sineroides*. Adult females feed mostly on cattle and rest in cattle sheds or haylofts from the end of July to the end of August or the beginning of September. For laboratory maintenance induced copulation can be used for this species. Males of the species move their claspers 14.5 times per one copulation on the average. Recently the species was successfully colonized by natural matings using continuous light of low intensity in a metal screened cage 30 cm^3 on a side.

DISCUSSION

Mosquitoes used in these studies were identified as *engarensis* on the basis of cytogenetic characters. Females collected in the field were allowed to deposit eggs, certain F_1 individuals set aside for morphological studies and the remaining siblings tested for hybridization and cytogenetic criteria used to define *engarensis*.

Although strains were collected from various places in Japan, Taiwan and Korea, the strains of *sinensis* from Urawa and Yungjyu were used because they had a wider range of morphological variations than others. The local morphological variations in *sinensis* have been reported in previous paper (Kanda and Oguma, 1976).

There are several unresolved problems concerning distribution records of some members of the *hyrcanus* species group on Hokkaido Island. Ever since described by Tsuzuki (1901), the name *yesoensis* still remains in question, whether it is a synonym of *sinensis* or a distinct species. The specimens observed by Tsuzuki (1901) might be *engarensis* if they could be checked for several morphological characters. While Yamada (1924) and Harrison (1973) treated *yesoensis* as a synonym of *sinensis*, Sasa and Kamimura (1971) raised some questions as to its identity with *sinensis*. Most of these troubles are certainly due to the incomplete description by Tsuzuki (1901) as well as to the lack of a typespecimen.

Two other members of the *hyrcanus* species group, *lesteri* and *sineroides* also exist in Hokkaido Island, as reported by Kamimura (1968), Ohtsuru and Ohmori (1961) and the present authors (to be published in a separate paper). Kamimura (1968), in addition, divided the eggs of the *hyrcanus* species group into three types using the deck types of the eggs. According to his report those three types were (1) a type with a wide deck, (2) one with a deck of intermediate width and (3) one with a very narrow deck. The present authors lean to White's interpretation (1977) of egg morphology. The form of eggs is sometimes so labile in response to either environmental (Deane and Causy, 1943; Ohtsuru and Ohmori, 1960) or genetic (Coluzzi, Cancrini and DiDeco, 1972) factors. Nearly all the known members of the extensive *hyrcanus* species group have distinctive eggs (Harrison and Scanlon, 1975). The present results showed that *sinensis* and *engarensis* have very close ratios of deck width to egg width. Ohtsuru et al. (1951) compared the ratios among *sinensis*, *lesteri*, *yatsushiroensis* and *sineroides* as follows; *sinensis* was .429 + .016, *lesteri* was .081 + .020, *yatsushiroensis* was .124 + .016 and *sineroides* was .152 + .039. Other data on egg measurements were also given and the differences in deck width and length of float were pointed out. Comparing those ratios of the members of the *hyrcanus* species group to that of *engarensis*, the latter is closer to *sinensis* than the others. The egg type of *engarensis*, therefore, might have been treated as that of *sinensis* in the past. Xu and Feng (1975) reported *kiangesensis*, *kweiyangensis* and *lesteri anthropophagus* along with the other well known members of the *hyrcanus* species group in China. Among those members only *sinensis* has a wide deck. In Korea *pullus* described by Yamada (1937) also has a narrow deck similar to *lesteri*. Therefore, *engarensis* is distinct from any other members of the species group.

The morphological characters described in the present paper may indeed be insufficient, by themselves, to warrant description of a new species, but the combination of morphology, reproductive isolation and a fixed inversion in 2R would point to the validity of *engarensis*.

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KEY TO FEMALES, PUPAE AND OVA OF *SINENSIS* AND *ENGARENSIS*

The following key should separate most females, pupae and ova of these two species, at least in Japan.

— Female palps with scattered pale scales between the pale bands; last two pale bands usually broad, sometimes fused. Costa of wing between base and subcostal pale spot usually with some scattered pale scales. Pupal wings ceases nearly always with rows of round dark spots. The ratio of width/length of lateral spines on VIIth abdominal segment is .22 ± .03. Egg with wide deck and the ratio of width of deck to width of egg is about.49. . . . . *sinensis*

— Female palps rarely with any scattered pale scales between the pale bands; last two pale bands narrow, not fused. Costa between base and subcostal pale spot rarely with any scattered pale scales. Pupal wing cases rarely with rows of dark spots. The ratio of width/length of lateral spines on VIIth abdominal segment is .24 ± .036. Egg with relatively wide deck, the ratio of width of deck to width of egg is about .36. . . . . *engarensis*

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TABLE 1. Comparisons of Morphological Characters between Adults of *A. sinensis* and *A. engarensis*

Palpal characters: Pale scales between the last two bands on palp

Locality (species)	No. observed	bands fused	wide	medium	narrow
URAWA *(sinensis)*	100	39	46	13	2
YUNGJYU *(sinensis)*	100	19	26	38	14
ENGARU *(engarensis)*	100	0	0	0	100*

*: with few pale scales in very few cases

Wing characters: Scales on humeral cross vein

Locality (species)	No. observed	(+)	(±)	(-)
URAWA *(sinensis)*	100	0	30	70
YUNGJYU *(sinensis)*	100	94	4	2
ENGARU *(engarensis)*	100	9	3	88

(+): 2 or more scales (±): 1 scale or one side bare (-); bare on both sides

Pale Scales on Costa between Base and Subcostal Pale Spot

Locality (species)	No. observed	none	very few	some	many	prominently
URAWA *(sinensis)*	100	5	33	52	3	7
YUNGJYU *(sinensis)*	100	1	15	37	42	5
ENGARU *(engarensis)*	100	94	6	0	0	0

Pale scales on Vein 1 between Subcostal and Preapical Pale Spots on Costa

Locality (species)	No. observed	none	very few	some	many	prominently
URAWA *(sinensis)*	100	0	60	17	3	20
YUNGJYU *(sinensis)*	100	2	0	8	33	57
ENGARU *(engarensis)*	100	14	0	0	0	86

A Black Mark or a Few Dark Scales on Extreme Base of Vein 5

Locality (species)	No. observed	dark mark	many	some	few or without
URAWA *(sinensis)*	100	35	3	37	25
YUNGJYU *(sinensis)*	100	63	11	15	11
ENGARU *(engarensis)*	100	100	0	0	0

TABLE 2. Pupal Differences

	No. observed	Mean No. of branches of setae on IV_5+V_5	Mean ratio of width/length of lateral spines VII	Dark spots on wing bag +	±	-
URAWA(*sinensis*)	100	46.0 ± 4.45	.220 ± .029	79	16	5
YUNGJYU(*sinensis*)	100	44.2 ± 5.97	.220 ± .025	86	10	4
ENGARU(*engarensis*)	100	37.4 ± 6.17	.240 ± .036	0	9	91

+: obvious; ± : exist but not obvious; -: not visible

TABLE 3. Larval Differences

	No. observed	Mean length of Ms4 setae	Branches of setae on $II_5 + V_5$
URAWA(*sinensis*)	100	38.6 ± 2.72	35.8 ± 3.23
YUNGJYU(*sinensis*)	100	23.3 ± 2.85	36.1 ± 2.68
ENGARU(*engarensis*)	100	23.9 ± 1.74	45.5 ± 4.37

TABLE 4. Differences in Ova (in microns)

	No. observed[oo]	Length of ova	Width of ova	Width of deck	Ratio of width (deck/ova)
URAWA(*sinensis*)	50	571.81 ± 22.90	187.86 ± 11.70	92.87 ± 7.70	.49 ± .038
ENGARU(*engarensis*)	50	560.44 ± 16.73	193.98 ± 9.10	69.19 ± 9.66	.36 ± .058

[oo]: ten ova of each of five batches were used.