Reproductive isolation among *Ae. riversi*, *Ae. alcasidi* and *Ae. scutellaris* of the *Aedes* (*Stegomyia*) *scutellaris* subgroup (Diptera: Culicidae)

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Abstract: The degree of sexual and reproductive isolation among three species of mosquitoes: *Aedes riversi* Bohart and Ingram, occurring in Okinawajima and Iriomotejima, Ryukyu Archipelago, and Tsushima, Kyushu, Japan; *Ae. alcasidi* Huang from Taiwan; and *Ae. scutellaris* (Walker) from Papua New Guinea, was evaluated by "hide-and-seek" tests and experimental crossings in the laboratory. The males of *Ae. riversi* from a given place could not discriminate females obtained from the same place. In a "hide-and-seek" test using *Ae. alcasidi* and *Ae. scutellaris*, all of *Ae. alcasidi* males which performed successful mating could discriminate conspecific females. The males of *Ae. riversi* and *Ae. scutellaris* could not readily discriminate conspecific females, and interspecific insemination took place. The crosses of *Ae. riversi* (Okinawa) females with *Ae. alcasidi* (Taiwan) males, *Ae. scutellaris* (Papua New Guinea) females with *Ae. riversi* (Iriomote or Tsushima) males, and *Ae. scutellaris* females with *Ae. alcasidi* males produced a large number of fertile hybrids. These hybrids were bred through to the F₃ or F₄ generation. The respective reciprocal cross was, however, completely sterile.

INTRODUCTION

Mosquito species of the *Aedes* (*Stegomyia*) *scutellaris* subgroup are widely distributed from tropical to temperate regions. As they comprise many insular populations, these mosquitoes provide ideal materials for study-

ing speciation and evolution. Among the members of the *Ae. scutellaris* subgroup are *Ae. riversi* Bohart and Ingram which inhabit the Ryukyu Archipelago and the islands north and west of Kyushu, Japan (Mogi, 1976), *Ae. alcasidi* Huang which occurs in Taiwan, and *Ae. scutellaris* (Walker) which occurs in Papua New Guinea. We report here the extent of cross-insemination and reproductive compatibility/incompatibility between different populations of *Ae. riversi*, and between *Ae. riversi*, *Ae. alcasidi* and *Ae. scutellaris*.

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MATERIALS AND METHODS

All mosquitoes used for the experiments were from colonies maintained in our laboratory. *Ae. riversi* of Okinawajima strain (OKI-riv) and Iriomotejima strain (IRI-riv) were originally obtained from eggs laid by females collected at Yona in the northern part of Okinawajima and at Komi in Iriomotejima, Ryukyu Archipelago, Japan, respectively. *Ae. riversi* of Tsushima strain (TSU-riv) was collected by Dr. M. Mogi in Tsushima, Japan, in 1978, while *Ae. alcasidi* Taiwan strain (TWN-alc) was collected by Dr. J. C. Lien at Taitung, Taiwan in 1972 and *Ae. scutellaris* of Papua New Guinea strain (PNG-scu) was collected by I. Miyagi at Port Moresby, Papua New Guinea in 1979.

The colonies as well as the mosquitoes used for each experiment were kept in a biotron with a controlled temperature of 26°C and 85% RH. A photoperiod of 16 hr of daylight was provided and a crepuscular period was simulated by gradual dimming to full darkness in a 30-min period. The dimming process was reversed in the morning. Larvae were reared in rectangular pans (41 cm in length, 29 cm in width and 5 cm in height) with 2,500 ml water at a density of 120/liter. The larvae were reared on a diet of powder mixed pellet for mouse and dried yeast (1:1). Each larval rearing pan was covered with a plastic sheet. In order to obtain virgin adults, pupae were separated according to size and were placed in groups of 10 to 15 per paper cup for emergence of the adults. The adults emerged 2 days after pupation. These groups with mixed sexes were discarded. The newly-emerged virgin adult females and males were placed in separate screen cages (20×20×30 cm). The adult rearing cages were provided with double layers of screen, and separated by a distance of 10 cm. Equal numbers of 50–100 females and males in cross-breeding and 50 females and males in back cross-breeding and controls were used in each cage. To obtain eggs, females were provided with a blood meal from an anesthetized mouse. Oviposition occurred about 3 days after the blood meal. In designating a cross, for example, *Ae. riversi* × *alcasidi*, the female is always

placed first. In hybrids, the abbreviation F₁ (*Ae. riversi* × *Ae. alcasidi*) stands for the F₁ hybrid produced by a cross between *Ae. riversi* female and *Ae. alcasidi* male.

1. *Insemination, hatching and adult surviving rates.* In order to know the daily insemination rate, 10 females and 10 males were removed from each cage on the 1st, 2nd, 3rd, 4th, 5th, 6th and 15th day after caging and the females were examined for the presence of sperms in the spermathecae. In the F₁ to F₄ hybrid self-crosses, the insemination rates were determined after a 3 to 25-day caging. In the controls, interstrain/ interspecific crosses and backcrosses, the insemination rates were determined after a 3-day caging. In order to obtain information concerning the fertility of eggs and viability of larvae and adults, all the eggs obtained in each interstrain/interspecific cross were utilized but 300 out of the eggs obtained in each control cage were selected at random. The rates of hatching for all experiments and emergence were recorded. As not all the embryonated eggs submerged in water always hatched out, 40 unhatched eggs out of the eggs submerged for 8 days were dissected and examined microscopically for embryonation. The dissected eggs were judged as embryonated if development had passed a 40-hr stage when ocelli and hairs were visible, and as non-embryonated (sterile) if either at an earlier stage or no embryonation was observed. Expression of the percentage of fertile eggs from the cross follows that given by Miyagi and Toma (1989). The adult survival rate for the number of larvae hatched was determined in each inter- and intra-specific cross.

2. *"Hide-and-see" test.* In order to know the ability of the males to inseminate, a single male of 2 or 3 days after emergence was put in a cage (20×20×30 cm) with 10 conspecific females of the same age for 24 hr. All females were dissected to determine the presence of sperms. The "hide-and-see" test (Hartberg and Craig, 1968) was applied to *Ae. riversi*, *Ae. alcasidi* and *Ae. scutellaris*. This experiment was designed to determine if each male of these species could readily discriminate a single conspecific female in the presence of a great excess of females of another species. The procedure follows that

given by Miyagi and Toma (1989). There were 5 replicates of the tests on OKI-*riv* with IRI-*riv* and OKI-*riv* with TWN-*alc*, and 22 replicates of the tests on TWN-*alc* with PNG-*scu*.

RESULTS

1. Insemination frequencies

In intraspecific and intrastrain matings, and interstrain matings in *Ae. riversi*, insemination rates were 70 to 100% after a 3-day caging (Table 1). Both of the interspecific crosses, OKI-*riv* × TWN-*alc* and its reciprocal cross, showed high insemination rates, 90 to 92.5%. The insemination rate obtained by the crosses, *Ae. riversi* × *Ae. scutellaris*, was 90% for IRI-*riv* and 35.5% for TSU-*riv* after a 3-day period but attained very high rates after a 15-day period. In the reciprocal

crosses, the rates were very low for IRI-*riv* and for TSU-*riv* but also attained high rates after prolonged caging. In the crosses of TWN-*alc* × PNG-*scu* and its reciprocal crosses, the rates were apparently low but increased gradually with prolongation of the caging.

2. Fertility of eggs obtained in interstrain/ interspecific crosses

In the control crosses, the insemination rate after a 3-day period was 100% in all three strains of *Ae. riversi*, but averaged 82.5% in TWN-*alc* and 87.5% in PNG-*scu*. The percentage of fertile eggs was more than 92.4% in all strains of *Ae. riversi*, 93.7% in TWN-*alc* and 71.8% in PNG-*scu* (Table 2). There were no significant differences in the insemination rate, the hatching rate and the percentage of fertile eggs among the

Table 1 Effect of mating period on the frequency of insemination in crosses among *Aedes riversi*, *Ae. alcasidi* and *Ae. scutellaris*.

Cross (♀×♂)	No. of replicates	% insemination of females on days after caging*						
		1	2	3	4	5	6	15
Control (50♀♀ and 50♂♂)								
OKI- <i>riv</i> ×OKI- <i>riv</i>	4	5.0	40.0	70.0	100.0			
IRI- <i>riv</i> ×IRI- <i>riv</i>	1			100.0				
TSU- <i>riv</i> ×TSU- <i>riv</i>	3			100.0				
TWN- <i>alc</i> ×TWN- <i>alc</i>	2	0	65.0	95.0	100.0			
PNG- <i>scu</i> ×PNG- <i>scu</i>	2	0	30.0	85.0	90.0	100.0		
Interstrain (100♀♀ and 100♂♂)								
OKI- <i>riv</i> ×IRI- <i>riv</i>	4			95.0				
IRI- <i>riv</i> ×OKI- <i>riv</i>	4			95.0				
IRI- <i>riv</i> ×TSU- <i>riv</i>	2			100.0				
TSU- <i>riv</i> ×IRI- <i>riv</i>	1			100.0				
Interspecies								
OKI- <i>riv</i> ×TWN- <i>alc</i> **	4			92.5				
TWN- <i>alc</i> ×OKI- <i>riv</i> **	2			90.0				
IRI- <i>riv</i> ×PNG- <i>scu</i> ***	4			90.0				100.0
PNG- <i>scu</i> ×IRI- <i>riv</i> ***	4			45.0				95.5
TSU- <i>riv</i> ×PNG- <i>scu</i> ***	2			35.5				90.0
PNG- <i>scu</i> ×TSU- <i>riv</i> ***	2			40.0				90.0
TWN- <i>alc</i> ×PNG- <i>scu</i> **	3	0	30.0	76.7	86.7	96.7	100.0	
PNG- <i>scu</i> ×TWN- <i>alc</i> **	3	0	3.0	53.3	93.3	96.7	100.0	

Abbreviation of strains: OKI-*riv*, *Ae. riversi* Okinawajima strain; IRI-*riv*, *Ae. riversi* Iriomotejima strain; TSU-*riv*, *Ae. riversi* Tsushima strain; TWN-*alc*, *Ae. alcasidi* Taiwan strain; PNG-*scu*, *Ae. scutellaris* Papua New Guinea strain. * Ten females were sequentially sampled and dissected on each day at each test. ** 100♀♀ and 100♂♂, *** 50♀♀ and 50♂♂.

Table 2 Egg fertility and adult emergence in crosses among *Aedes riversi*, *Ae. alcasidi* and *Ae. scutellaris*.

Cross (♀ × ♂)	No. of replicates	% insemination of females ¹⁾	No. of eggs examined	% of eggs			% of adults emerged ⁴⁾
				Hatched	Embryonated ²⁾	Fertile ³⁾	
OKI- <i>riv</i> × OKI- <i>riv</i>	4	100.0	1,200	86.0	55.4	93.8	95.4
IRI- <i>riv</i> × IRI- <i>riv</i>	1	100.0	343	82.2	57.1	92.4	77.0
TSU- <i>riv</i> × TSU- <i>riv</i>	3	100.0	904	75.7	89.4	97.5	73.8
TWN- <i>alc</i> × TWN- <i>alc</i>	4	82.5	1,342	75.6	74.8	93.7	88.8
PNG- <i>scu</i> × PNG- <i>scu</i>	2	87.5	595	68.2	8.7	71.8	100.0

Fifty females and 50 males were caged together. ¹⁾ Insemination rate was determined after a 3-day mating period. ²⁾ Percentage of embryonated eggs out of 40 unhatched eggs. ³⁾ % of fertile eggs = {(No. of eggs hatched + No. of unhatched but embryonated eggs) / No. of eggs examined} × 100. ⁴⁾ Percentage of mosquitoes emerged into adult from 1st instar larvae. For abbreviation of strains, see footnote of Table 1.

Table 3 Egg fertility and adult emergence in interstrain crosses of *Aedes riversi*.

Cross (♀ × ♂)	Genera- tion	No. of replicates	% insemination of females	No. of eggs examined	% of eggs			% of adults emerged
					Hatched	Embryo- nated	Fertile	
OKI- <i>riv</i> × IRI- <i>riv</i>	F ₁	4	100.0 (12)*	1,172	89.7	68.6	96.8	86.7
	F ₂	2	100.0 (5)	584	91.8	80.0	97.8	86.9
	F ₃	2	100.0 (11)	602	93.4	50.0	95.8	96.8
IRI- <i>riv</i> × OKI- <i>riv</i>	F ₁	4	97.5 (12)	1,215	88.1	61.0	96.4	84.8
	F ₂	2	100.0 (5)	574	86.9	90.3	98.6	81.6
	F ₃	2	100.0 (11)	572	88.6	66.7	96.0	93.7
IRI- <i>riv</i> × TSU- <i>riv</i>	F ₁	2	100.0 (12)	570	84.4	75.0	95.8	95.2
TSU- <i>riv</i> × IRI- <i>riv</i>	F ₁	2	100.0 (12)	287	82.6	45.5	90.6	91.6

One hundred females and 100 males were caged together. For calculations of % embryonation, % fertility and % adult emergence, see footnote of Table 2. For abbreviation of strains, see footnote of Table 1. * Figure in bracket shows the mating period in days when insemination rate was determined.

interstrain crosses of *Ae. riversi* (Table 3) and the control (Table 2).

In the interspecific cross, OKI-*riv* × TWN-*alc*, the average insemination rate after a 3-day caging was 80 to 100% with 84.9 to 94.2% fertile eggs in F₁ to F₃ generations. While in the reciprocal cross, there were no fertile eggs (Table 4).

In the crosses IRI-*riv* or TSU-*riv* × PNG-*scu*, none of the parental females produced fertile eggs; but in the reciprocal crosses, females of both strains, however, produced 60.7 to 84.0% fertile eggs. In the cross TWN-*alc*

× PNG-*scu*, there were no fertile eggs; but in the reciprocal cross, 75.1 to 96.3% fertile eggs were obtained in F₁ to F₄ generations.

3. Backcrosses to parental species

All the backcrosses of F₁(OKI-*riv* × IRI-*riv*) hybrids and F₁(IRI-*riv* × OKI-*riv*) hybrids to the parental strains of *Ae. riversi* were successful (Table 5). The insemination and hatching rates of the backcrosses were similar to those in the parental strains (Tables 2, 5).

As shown in Table 6, all the backcrosses

Table 4 Egg fertility and adult emergence in crosses among *Aedes riversi*, *Ae. alcasidi* and *Ae. scutellaris*.

Cross (♀ × ♂)	Genera- tion	No. of replicates	% insemi- nation of females	No. of eggs examined	% of eggs			% of adults emerged
					Hatched	Embryo- nated	Fertile	
OKI-riv × TWN-alc	F ₁	4	97.5 (3) *	1,237	79.1	64.4	92.2	94.2
	F ₂	2	80.0 (3)	571	83.0	69.2	94.2	83.5
	F ₃	2	100.0 (17)	616	70.8	48.5	84.9	95.9
TWN-alc × OKI-riv	F ₁	2		6,600	0.0	0.0	0.0	0.0
IRI-riv × PNG-scu	F ₁	2		4,123	0.0	0.0	0.0	0.0
PNG-scu × IRI-riv	F ₁	2		3,551	69.0	35.5	81.5	84.5
TSU-riv × PNG-scu	F ₁	2		5,102	0.0	0.0	0.0	0.0
PNG-scu × TSU-riv	F ₁	2	85.0 (3)	4,129	52.6	16.5	60.7	86.1
	F ₂	1	100.0 (14)	239	60.7	67.6	84.0	83.4
TWN-alc × PNG-scu	F ₁	3		6,600	0.0	0.0	0.0	0.0
PNG-scu × TWN-alc	F ₁	3	100.0 (12)	6,948	80.0	0.0	80.0	97.5
	F ₂	3	93.3 (25)	11,900	93.8	58.3	96.3	80.8
	F ₃	3	100.0 (8)	6,643	62.7	32.5	75.1	94.7
	F ₄	3		900	87.8	34.9	91.9	89.2

One hundred females and 100 males were caged together. For calculations of % embryonation, % fertility and % adult emergence, see footnote of Table 2. For abbreviation of strains, see footnote of Table 1. * Figure in bracket shows the mating period in days when insemination rate was determined.

Table 5 Egg fertility and adult emergence in reciprocal backcrosses of F₁ hybrids to parental strains of *Aedes riversi*.

Cross (♀ × ♂)	% insemi- nation of females*	No. of eggs examined	% of eggs			% of adult emerged
			Hatched	Embryonated	Fertile	
OKI-riv × F ₁ (OKI-riv × IRI-riv)	100.0	301	91.0	81.3	98.3	89.8
F ₁ (OKI-riv × IRI-riv) × OKI-riv	100.0	294	87.8	80.0	97.6	86.4
IRI-riv × F ₁ (OKI-riv × IRI-riv)	100.0	318	89.6	71.4	96.9	88.1
F ₁ (OKI-riv × IRI-riv) × IRI-riv	100.0	345	92.8	81.8	98.8	86.3
IRI-riv × F ₁ (IRI-riv × OKI-riv)	100.0	321	92.2	78.6	98.4	80.1
F ₁ (IRI-riv × OKI-riv) × IRI-riv	100.0	296	88.2	93.3	99.3	89.3
OKI-riv × F ₁ (IRI-riv × OKI-riv)	100.0	321	95.9	100.0	100.0	89.0
F ₁ (IRI-riv × OKI-riv) × OKI-riv	100.0	317	92.4	88.9	99.1	93.5

All backcrosses were carried out with 50 females and 50 males. For calculations of % embryonation, % fertility and % adult emergence, see footnote of Table 2. For abbreviation of strains, see footnote of Table 1. * Insemination rate was determined after a 3-day mating period.

Table 6 Egg fertility and adult survival rates in reciprocal backcrosses of F_1 hybrids to parental species.

Cross (♀ × ♂)	% insemination of females*	No. of eggs examined	% of eggs			% of adult emerged
			Hatched	Embryonated	Fertile	
OKI-riv × F_1 (OKI-riv × TWN-alc)	100.0	613	94.0	66.7	98.0	87.0
F_1 (OKI-riv × TWN-alc) × OKI-riv	100.0	702	86.3	76.1	96.6	88.0
TWN-alc × F_1 (OKI-riv × TWN-alc)	80.0	660	0.0	0.0	0.0	0.0
F_1 (OKI-riv × TWN-alc) × TWN-alc	100.0	596	82.6	71.7	95.1	88.4
PNG-scu × F_1 (PNG-scu × TWN-alc)	100.0	4,950	80.6	2.9	81.1	82.3
F_1 (PNG-scu × TWN-alc) × PNG-scu	100.0	5,809	88.3	56.5	93.9	95.8
TWN-alc × F_1 (PNG-scu × TWN-alc)	100.0	8,449	0.0	0.0	0.0	0.0
F_1 (PNG-scu × TWN-alc) × TWN-alc	100.0	5,102	88.7	39.1	91.7	84.0

All backcrosses were carried out with 50 females and 50 males. For calculations of % embryonation, % fertility and % adult emergence, see footnote of Table 2. For abbreviation of strains, see footnote of Table 1. * Insemination rate was determined after a 3-day mating period.

Table 7 Number of females inseminated from a single conspecific male during a 24-hr period.

Species	No. of tests	No. of cases						
		No. of inseminated females in a cage						
		1	2	3	4	5	6	7-10
OKI-riv	5	0	0	0	2	3	0	0
IRI-riv	5	1	0	0	0	4	0	0
TWN-alc	9	2	1	0	3	2	1	0
PNG-scu	5	3	0	2	0	0	0	0

A single male was caged with 10 conspecific females. For abbreviation of strains, see footnote of Table 1.

except TWN-alc × F_1 (OKI-riv × TWN-alc) and TWN-alc × F_1 (PNG-scu × TWN-alc) were successful.

4. "Hide-and-seek" test

Each male of *Ae. riversi*, *Ae. alcasidi* and *Ae. scutellaris* usually inseminated 4 or 5 females of the same species but did not inseminate more than 6 females during a 24-hr period (Table 7).

As shown in Table 8, in the "hide-and-seek" test, one OKI-riv male was placed with one OKI-riv female and 19 IRI-riv females; likewise, one IRI-riv male was placed with one IRI-riv female and 19 OKI-riv females. None of the males discriminated females of their own species. In the test with OKI-riv and TWN-alc, males of OKI-riv did not discriminate their own females, and inseminated

females of the other species. Sixty percent of TWN-alc males could discriminate their own females, but the males also inseminated the other species. The test with TWN-alc and PNG-scu was replicated 22 times. All of TWN-alc males which performed mating could discriminate their own females, and none of the 19 PNG-scu females in each cage tested were inseminated by TWN-alc males. PNG-scu males did not discriminate their own females and inseminated TWN-alc females.

DISCUSSION

The *Aedes scutellaris* group of the subgenus *Stegomyia* is divided into two subgroups, the *Ae. albopictus* subgroup and the *Ae. scutellaris* subgroup. The species of the latter sub-

Table 8 "Hide-and-seek" test with one male mosquito caged with one homotypic female and 19 heterotypic females.

Combination*	No. of replicates	No. of cases										
		Homotypic female	No. of heterotypic females inseminated									
			0	1	2	3	4	5	6	7	8-19	
1	5	Inseminated	1	0	0	1	0	0	0	0	0	0
		Uniseminated	4	0	0	1	1	1	1	0	0	0
2	5	Inseminated	3	0	0	0	0	2	0	0	1	0
		Uniseminated	2	0	0	0	1	0	0	1	0	0
3	5	Inseminated	0	0	0	0	0	0	0	0	0	0
		Uniseminated	5	0	0	0	0	4	1	0	0	0
4	5	Inseminated	3	0	0	2	1	0	0	0	0	0
		Uniseminated	2	0	0	0	2	0	0	0	0	0
5	22	Inseminated	12	12	0	0	0	0	0	0	0	0
		Uniseminated	10	10	0	0	0	0	0	0	0	0
6	22	Inseminated	0	0	0	0	0	0	0	0	0	0
		Uniseminated	22	17	2	1	2	0	0	0	0	0

For abbreviation of strains, see footnote of Table 1. * Combination: 1, OKI-*riv* male, OKI-*riv* female and IRI-*riv* females; 2, IRI-*riv* male, IRI-*riv* female and OKI-*riv* females; 3, OKI-*riv* male, OKI-*riv* female and TWN-*alc* females; 4, TWN-*alc* male, TWN-*alc* female and OKI-*riv* females; 5, TWN-*alc* male, TWN-*alc* female and PNG-*scu* females; 6, PNG-*scu* male, PNG-*scu* female and TWN-*alc* females.

group are widely distributed in Southeast Asia, South Pacific islands, Papua New Guinea, and Japan (Kyushu and Ryukyu Archipelago). Many endemic species and subspecies of this subgroup have been described, especially from different islands, and, therefore, they offer interesting subjects for studies in speciation of mosquitoes. *Ae. riversi* and *Ae. alcasidi* are Southeast Asian species, while *Ae. scutellaris* is a Papua New Guinean species of the *Ae. scutellaris* subgroup. They are extremely variable morphologically and difficult to separate in all stages except for the male terminalia.

Ae. riversi is a troublesome biter which is common at foothill forests in the Ryukyu Archipelago and the southern part of Kyushu, Japan (Tsushima, Fukuejima, Danjo Is., Cape Sata and Yakushima), but very few individuals have been found near human dwelling (Toma and Miyagi, 1981, 1986; Mogi, 1976). The immatures have been commonly collected from tree holes and arti-

ficial containers discarded in forests.

Ae. alcasidi was described by Huang (1972) based on the specimens from the Philippines and has been mistaken in the past for *Ae. scutellaris* (Knight and Hull, 1952) and for *Ae. malayensis* (Colless, 1962). Huang (1972) recorded *Ae. malayensis* Colless from Orchid Island (Lanyu), Taiwan. However, Matsuo *et al.* (1974) suggested that the adult specimens of the *Ae. scutellaris* subgroup both from Lanyu Is. and Taiwan proper south of 23°N latitude should be identified as *Ae. alcasidi*. Huang (1973) recorded this species from Sabah, Malaysia. As far as we know, this species is distributed in the Philippines (Luzon), Taiwan including Lanyu Is. and East Malaysia (Sabah). The immature stages of *Ae. alcasidi* have been collected mainly in tree holes and bamboo stumps and were associated with *Ae. albopictus* in the Philippines and Taiwan (Huang, 1972; Matsuo *et al.*, 1974).

Ae. scutellaris is complicated taxonomical-

Fig. 1 Results of experimental crosses between *Ae. riversi* from Okinawajima, Iriomotejima and Tsushima, *Ae. alcasidi* from Taiwan and *Ae. scutellaris* from Papua New Guinea.

ly. It comprises three subspecies: *Ae. s. scutellaris* (Walker) from Ceram, Ambon, Aru Islands and New Guinea; *Ae. s. malayensis* Colless from Singapore, Malaysia, Thailand, Cambodia and Vietnam; and *Ae. s. katherinensis* Woodhill from Australia

(Knight and Stone, 1977). *Ae. s. malayensis* was originally described as a subspecies by Colless (1962) from Singapore, but Huang (1972) elevated it as a distinct species, *Ae. malayensis*. However, it has been treated as a subspecies of *Ae. scutellaris* by Knight and

Stone (1977).

The results of this study show that the Okinawajima, Iriomotejima and Tsushima strains of *Ae. riversi* are completely interfertile. It is evident that these strains are conspecific, because the F₁ hybrid progenies produced by these strains were completely compatible with the parent strains (Fig. 1).

The sterility barrier between *Ae. alcasidi* females and males of *Ae. riversi* Okinawajima strain was complete, so was that between *Ae. scutellaris* males and females of *Ae. riversi* Iriomotejima and Tsushima strains (Fig. 1). The cross between *Ae. alcasidi* females and *Ae. scutellaris* males was also completely sterile. On the other hand, the hybrid females obtained by crossing *Ae. riversi* females with *Ae. alcasidi* males were fertile when backcrossed with males of *Ae. riversi* or *Ae. alcasidi*. The hybrid males produced by this cross (*Ae. riversi* females and *Ae. alcasidi* males) were only fertile when backcrossed with *Ae. riversi* females. However, the hybrid males obtained by crossing *Ae. scutellaris* females with *Ae. alcasidi* males were sterile when backcrossed with *Ae. alcasidi* females.

Dev and Rai (1985) showed that *Ae. scutellaris katherinensis* from the Northern Territory, Australia, and *Ae. alcasidi* from Taiwan were bidirectionally compatible and the percentage of egg hatch was different in reciprocal crosses. It was relatively higher with *Ae. s. katherinensis* as the parental female (77% egg hatch), than with *Ae. alcasidi* (53% egg hatch). The hybrids obtained by crossing *Ae. alcasidi* females with *Ae. s. katherinensis* males were equally fertile when selfed, and when backcrossed to either parent. The hybrid females obtained by crossing *Ae. s. katherinensis* females with *Ae. alcasidi* males were also fertile when backcrossed with males of *Ae. alcasidi* or *Ae. s. katherinensis*. However, the sterility barrier between *Ae. s. katherinensis* males from Australia and *Ae. malayensis* females from Thailand was complete, and the reciprocal cross (*Ae. malayensis* males × *Ae. s. katherinensis*), showed an intermediate degree of mating compatibility resulting in 63% mean egg hatch (Dev and Rai, 1985).

The results of the present study on "hide-and-seek" test and experimental crossing show that *Ae. riversi*, *Ae. alcasidi* and *Ae.*

scutellaris are sexually and reproductively isolated. The present findings of incompatibility between *Ae. alcasidi* females and *Ae. s. scutellaris* males and Dev and Rai's findings (Dev and Rai, 1985) of bidirectional compatibility between *Ae. alcasidi* and *Ae. s. katherinensis* indicate that incompatibility probably occurs between *Ae. s. scutellaris* (Papua New Guinea) and *Ae. s. katherinensis*. Experimental crossings of *Ae. alcasidi* and *Ae. s. malayensis*, *Ae. s. malayensis* and *Ae. scutellaris*, and *Ae. scutellaris* (Papua New Guinea) and *Ae. s. katherinensis* will throw more light on the speciation of the *Ae. scutellaris* subgroup.

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摘 要

Aedes (Stegomyia) scutellaris subgroup
に属する *Ae. riversi*, *Ae. alcasidi* と
Ae. scutellaris の生殖隔離

地理的に異なるリバーズシマカ *Ae. riversi* Bohart and Ingram の3系統 (沖縄島, 西表, 対馬) 間, および台湾産 *Ae. alcasidi* Huang, パプアニューギニア産 *Ae. scutellaris* (Walker) の3種間の生殖隔離の程度を明らかにする目的で, 室内で交雑実験を行った。

リバーズシマカ3系統間の組合せでは, いずれも自由に交尾, 受精し, 生殖隔離はみられなかった。リバーズシマカ雌 (沖縄島産) と *Ae. alcasidi* 雄, *Ae. scutellaris* 雌とリバーズシマカ雄 (西表島・対馬産), *Ae. scutellaris* 雌と *Ae. alcasidi* 雄の組合せは受精が成立し, 多数の雑種 (F₃ または F₄) が得られた。また, これらの逆の組合せでは, 交尾は行い, 貯精嚢に精子は入るが, まったく受精は成立せず, 雑種を得ることはできなかった。