Dissertation Abstract

Studies of coexistence mechanism between *Aedes albopictus* and *Aedes flavopictus*
ヒトスジシマカとヤマダシマカの２種の共存機構の研究

By
SULTANA AFROZA

Graduate School of Natural Science & Technology
Kanazawa University

Division of Natural system

Abstract

Aedes albopictus and *Aedes flavopictus* are ecologically similar that have overlapped distribution. To know the coexistence, we studied the reproductive interference. Here, we tested the effect of body size on copulation time, fecundity and hatchability after conspecific, heterospecific and double-mating. Females mated with heterospecific males produced sterile eggs. Reproductive interference had negative effects on *Ae. flavopictus* than *Ae. albopictus* females. Larger females were less affected by heterospecific copulation, but the processes were different to reduce the effects of heterospecific mating. Vegetation act as a driving factor for the growth of *Ae*. *albopictus*. Larvae raised by bamboo resulted in large body size and higher fecundity, however, cherry leaf had an antagonistic effect. Besides, body size had a greater impact on the fecundity, however, *Ae. albopictus* laid more eggs than *Ae*. *flavopictus*. Notably, there was no significant relation ($P>0.05$) between body size and longevity. Furthermore, both species were susceptible to temperature and humidity. Higher temperature (28ºC) and lower humidity (52%) hindered fecundity and longevity of *Ae. flavopictus*. Conversely, *Ae. albopictus* exposed to similar condition showed better fecundity but had the negative effect on longevity. Overall, varying body size of females exhibited difference in mating, fecundity and longevity that facilitated the coexistence.

1. Introduction

Aedes albopictus and *Aedes flavopictus* are sibling species belonging to subgenus *Stegomyia* (Tanaka et al. 1979). *Ae. albopictus* is extended its distribution to Africa, Europe, Australia, America, and Middle East (Gratz 2004, Benedict et al. 2007), however, *Ae. flavopictus* has a limited distribution only in Japan and Korea. Furthermore, *Ae*. *albopictus* occurs from the Ryukyu islands in Tohoku district, while *Ae*. *flavopictus* is dispersed throughout Japan, including Hokkaido region. *Ae*. *albopictus* and *Ae*. *flavopictus* species overlap their distribution; the former mainly inhabits in urban and semi-urban areas, whereas the latter does in forests areas (Tanaka et al. 1979, Sota et al. 1992). However, they are expected to encounter in breeding habitats, because their habitation preferences are not rigid (Sunahara et al. 2002). Despite habitat choice, it is crucial to know what other factors influence the coexistence of these two species? Therefore, we consider reproductive interference may be the most plausible reason of the co-occurrence between these species.

Reproductive interference with the larval resource competition may permit cohabitation and distribution of species. The confront of species may be mediated by the choice of breeding habitats, where larvae feed on leaf detritus from different vegetation. To address this, there is an urge to elucidate the role of different vegetation that interact with the development of *Ae. albopictus*. Furthermore, climate change seems to be the driving factors that may allow the distribution of *Ae. albopictus* and *Ae. flavopictus*. Mosquito activity, development, mortality, reproduction and longevity are affected by temperature, relative humidity and precipitation (Costa et al. 2010, Roiz et al. 2014, Danis-Lozano et al. 2015, Panackal 2016, Khan et al. 2018, Drakou et al. 2020). In a changing climatic condition, differences in the temperature and food availability result in different body size of adults that influence on the fecundity and longevity. The transmission of vector-borne disease is strongly linked to body size that affects the female's vectorial capacity (Christiansen-Jucht et al. 2015, Barreaux et al. 2018). Particularly, our study aimed to evaluate the following points: 1. Reproductive interference between *Aedes albopictus* and *Aedes flavopictus* 2. Vegetation influence on *Ae. albopictus* 3. Influence of body size on the fecundity and longevity 4. Impact of temperature and humidity on the fecundity and longevity.

2. Materials and Methods

The present study was conducted in the laboratory of Ecology during the period from 2018 to 2019, Kanazawa University, Japan.

3. Results and Discussion

3.1. Reproductive interference between *Aedes albopictus* and *Aedes flavopictus* at a place of their origin

In heterospecific mating, females of both species produced sterile eggs. *Ae. flavopictus* copulated longer during heterospecific mating which adversely affect the remating with conspecific males, as well as causing severe loss of fecundity and hatching. Considerably, the asymmetric effects of heterospecific mating between two species favor *Ae. flavopictus* due to the larger body size. The body size of *Ae. flavopictus* can have a significant effect on the reproductive interference resulted in the dominance of *Ae. albopictus* as well as *Ae. flavopictus* did not replace by *Ae. albopictus*. From this evidence, it is clear that both species would continue to coexist in Japan by differentiating landscape level distributions.

3.2. Effect of vegetation on the larval and adult growth performance of *Aedes albopictus* (Diptera: Culicidae)

The highest larval mortality was observed in cherry and beech, however, the lowest in bamboo. Larval stages feed upon cherry and beech showed delay in development and adult emergence than that of bamboo. Female body size was larger when larvae raised with the bamboo compared to cherry plant. *Ae. albopictus* females oviposited more eggs in bamboo vegetation, however, adult nurtured by cherry plants laid less amount of eggs. Per capita performance of *Ae. albopictus* on bamboo plants was higher for the larval and adult growth compared to cherry and beech. Thus, *Ae. albopictus* larvae were affected by bamboo vegetation that might have influenced on the larval growth and attracted adult mosquitoes for oviposition.

3.3. Influence of body size on the fecundity and longevity of *Aedes albopictus* and *Aedes flavopictus*

The body size of *Ae. flavopictus* was larger than that of *Ae. albopictus* in all temperatures (25°C and 28°C) and diets (low and high) treatments. *Ae. flavopictus* retained more eggs compared to *Ae. albopictus* however, body size and egg retention were negatively correlated. In both species, body size showed a positive correlation with the number follicles. As fecundity increased with the body size, *Ae. albopictus* laid more eggs than *Ae. flavopictus*. Conversely, *Ae. flavopictus* retained more eggs compared to *Ae. albopictus*. Notably, there was no significant relation ($P>0.05$) between the body size and longevity. Thus, the impact of body size on fecundity might be considered to control vector.

3.4. Temperature and humidity influence on the fecundity and longevity of *Aedes albopictus* and *Aedes flavopictus*

Oviposition time of both species extended as relative humidity increased (52%RH) at low temperature (25ºC). Conversely, higher temperature and lower humidity reduced the hatching of both species. Higher temperature (28ºC) and lower humidity (47%RH) hindered the fecundity and longevity of *Ae. flavopictus*. However, *Ae. albopictus* exposed to those atmospheric conditions exhibited better fecundity with the deleterious effect on longevity. So, this study enabled to determine suitable environmental condition for vector mosquito that might help to make reliable predictions of disease transmission.

Conclusion

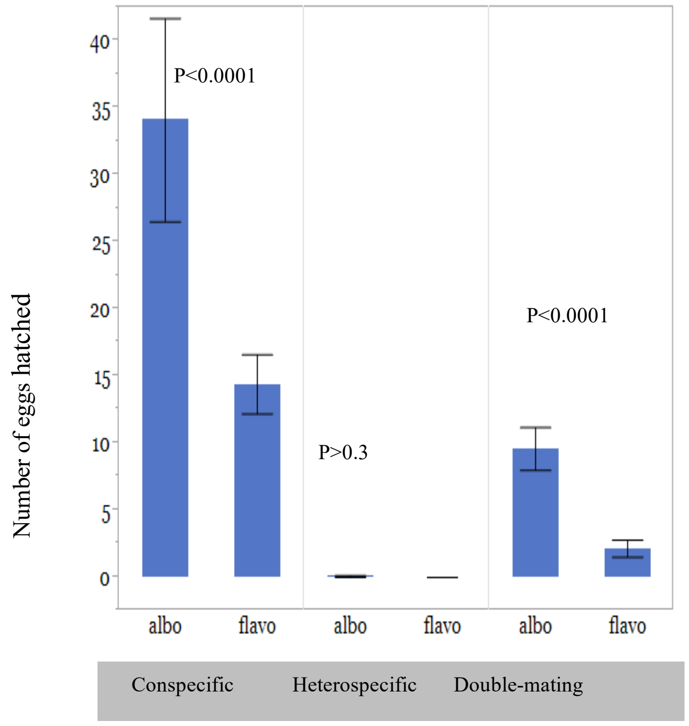
Ae. albopictus is now distributed throughout the world, causing the elimination of related *Aedes* species. However, it has long been cohabiting with *Ae. flavopictus* in Japan. Overall, substantial differences in the mating biology, fecundity and longevity of ecologically similar species that facilitated the coexistence of *Ae. albopictus* and *Ae. flavopictus*.

Table1. Wing length (mm) (Mean ± SD) of females and males, copulation duration (Sec) (Mean ± SD), numbers of eggs oviposited (Mean ± SD) and hatched per female and hatching rates of eggs in conspecific (C), heterospecific (I) and double-mating (D: heterospecific mating followed by conspecific mating) experiments with *Aedes albopictus* (A) and *Aedes flavopictus* (F). L: large individuals reared at 22°C, S: small individuals reared at 28°C.

				Male (first mating)			Male (second mating)			Copulation duration				
Mating	Female	N	Wing length		N	Wing length		N	Wing length	first mating	second mating	Number of of eggs oviposited	Number of eggs hatched	Hatching rate of eggs
C	A (L)	40	3.16 ± 0.08	A (S)	20	2.23 ± 0.09	-	-	-	155.6 ± 29.4	-	33.7 ± 30.1	33.7 ± 30.1	1
C	A (S)	40	2.87 ± 0.18	A (L)	20	2.58 ± 0.07	-	-	-	146.6 ± 31.2	-	35.4 ± 38.9	34.6 ± 38.1	0.98
C	F (L)	40	3.61 ± 0.14	F (S)	20	2.47 ± 0.10	-	-	-	175.8 ± 28.6	-	17.2 ± 10.8	16.0 ± 10	0.93
C	F (S)	40	3.24 ± 0.13	F (L)	20	2.78 ± 0.08	-	-	-	127.1 ± 22.5	-	14.7 ± 9.3	12.8 ± 9.2	0.87
I	A (L)	40	3.14 ± 0.09	F (S)	20	2.47 ± 0.09	-	-	-	45.1 ± 25.3	-	0.4 ± 1.1	0	0
I	A (S)	40	2.89 ± 0.15	F (L)	20	2.79 ± 0.11	-	-	-	147.0 ± 42.3	-	8.1 ± 14.2	0.1 ± 0.5	0.01
I	F (L)	40	3.60 ± 0.13	A (S)	20	2.29 ± 0.13	-	-	-	164.5 ± 68.7	-	0.8 ± 1.7	0	0
I	F (S)	40	3.29 ± 0.16	A (L)	20	2.59 ± 0.08	-	-	-	118.2 ± 43.5	-	2.6 ± 4.4	0	0
D	A (L)	40	3.17 ± 0.10	F (S)	20	2.48 ± 0.12	A (S)	20	2.27 ±0.09	55.3 ± 14.2	161.3 ± 54.2	11.2 ± 6.9	10.5 ± 6.8	0.94
D	A (S)	40	2.89 ± 0.15	F (L)	20	2.75 ± 0.14	A (L)	20	2.60 ± 0.10	144.1 ± 38.7	123.3 ± 45.4	10.0 ± 7.7	8.6 ± 7.5	0.86
D	F (L)	40	3.61 ± 0.11	A (S)	20	2.25 ± 0.14	F (S)	20	2.44 ± 0.10	150.4 ± 60.4	130.9 ± 46.4	5.1 ± 3.6	3.5 ± 3.3	0.69
D	F (S)	40	3.26 ± 0.16	A (L)	20	2.61 ± 0.15	F (L)	20	2.80 ± 0.10	113.9 ± 43.5	84.1 ± 40.4	3.4 ± 3.2	0.8 ± 1.4	0.24

Table 2. Developmental time from the first instar to adult emergence (day), female wing size (mm), number of eggs laid per female in one gonotrophic cycle of *Aedes albopictus* raised with different vegetation with pairwise test results (Tukey Kramer honestly significant different test).

		Adult development time (day)			Female wing size (mm)			Number of eggs	
Temperature	Vegetation type	n1	(Mean ± SD)	HSD test*	n2	(Mean ± SD)	HSD test*	(Mean ± SD)	HSD test*
			F= 242.4, P<0.0001			F= 62.84, P<0.0001		F= 2.91, P= 0.06	
22°C	Bamboo	100	28.5 ± 2.35	A	20	3.10 ± 0.08	A	21.3 ± 11.7	A
	Cherry tree	100	40.2 ± 3.98	B	20	2.94 ± 0.06	B	13.6 ± 7.40	B
	Beech tree	100	34.9 ± 4.53	C	20	3.07 ± 0.07	C	20.2 ± 12.9	C
			F=43.52, P<0.0001			F= 25.11, P<0.0001		F= 1.12, P= 0.33	
28°C	Bamboo	100	18.0 ± 1.85	a	20	2.50 ± 0.07	a	26.1 ± 14.2	a
	Cherry tree	100	20.0 ± 4.35	b	20	2.38 ±0.07	b	19.3 ± 13.0	b
	Beech tree	100	23.5 ± 5.59	c	20	2.49 ± 0.04	c	23.2 ± 15.6	c



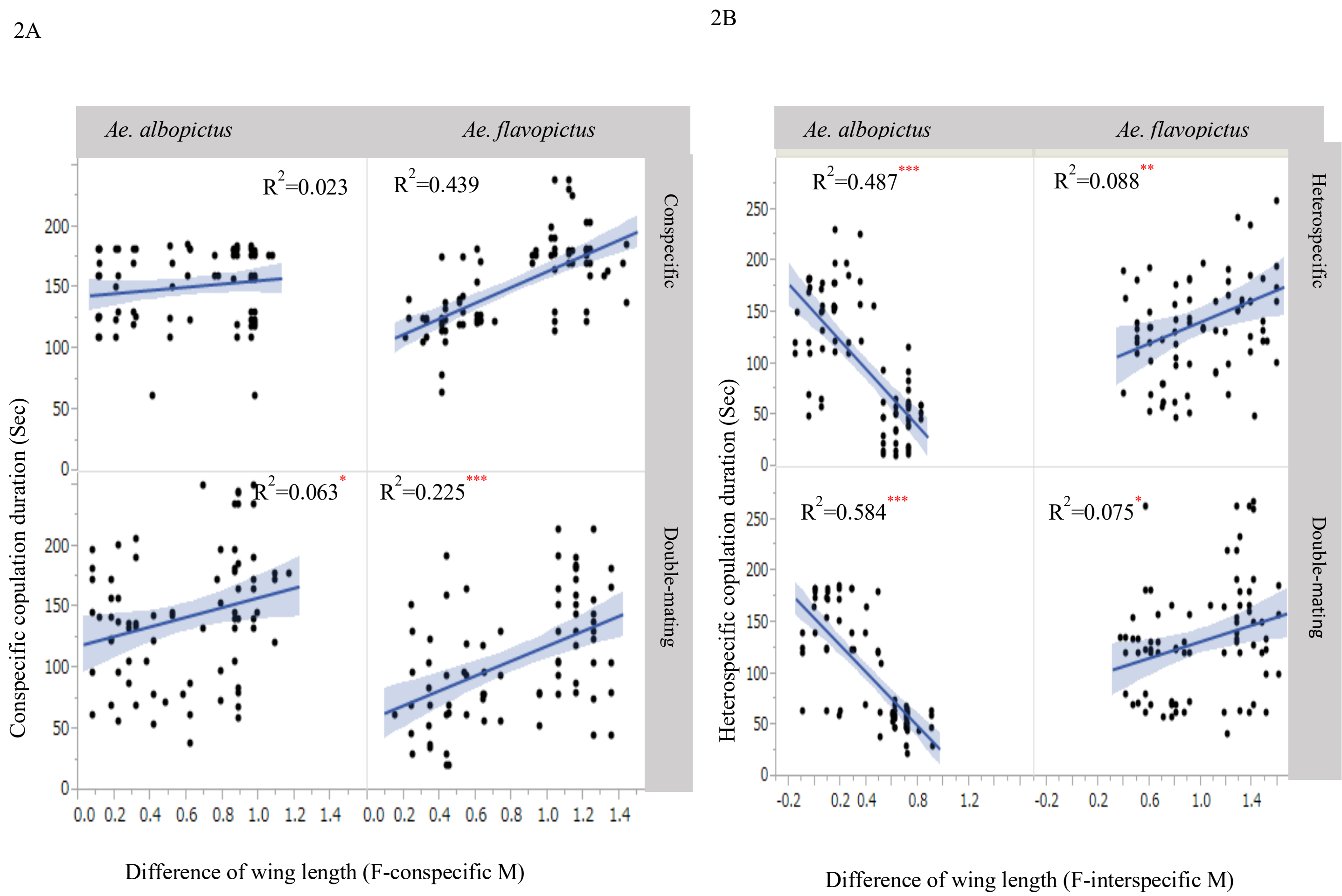


Fig. 1. Number of eggs hatched of *Aedes albopictus* (albo) and *Aedes flavopictus* (flavo) in the three mating experiments with results of species-pairwise comparisons.

Fig. 2. Regression analysis between difference of female wing length and conspecific male wing length and conspecific copulation duration (Fig. 2A); Regression analysis between difference of female wing length and different species male wing length and heterospecific copulation duration (Fig. 2B).

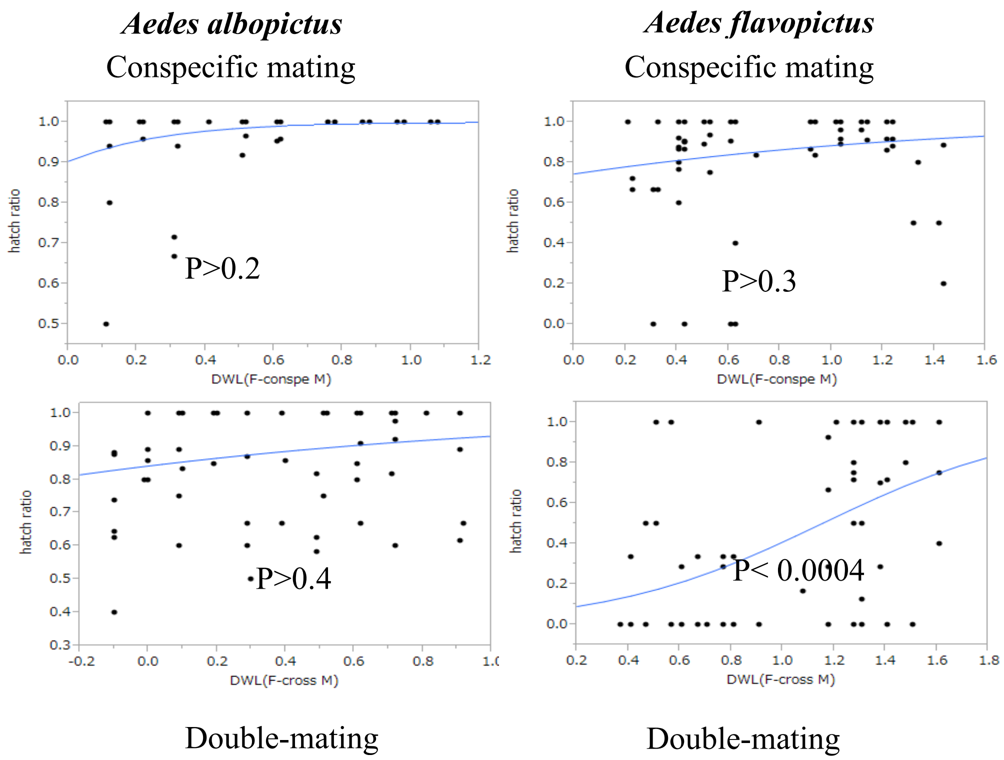


Fig. 3. The relationship between egg hatch ratio and difference of female wing length in *Ae. albopictus* and *Ae. flavopictus* in conspecific and double-mating experiments.

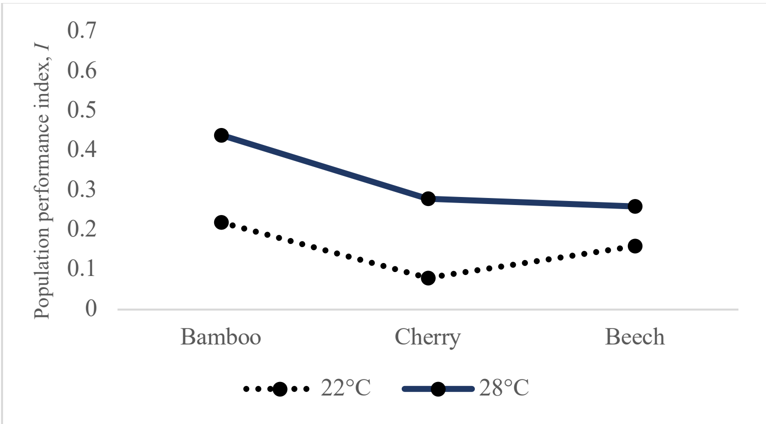


Fig. 4. Estimated population performance index (*I*) values of *Ae. albopictus* were reared under three vegetation treatments with two temperatures at 22°C and 28°C.

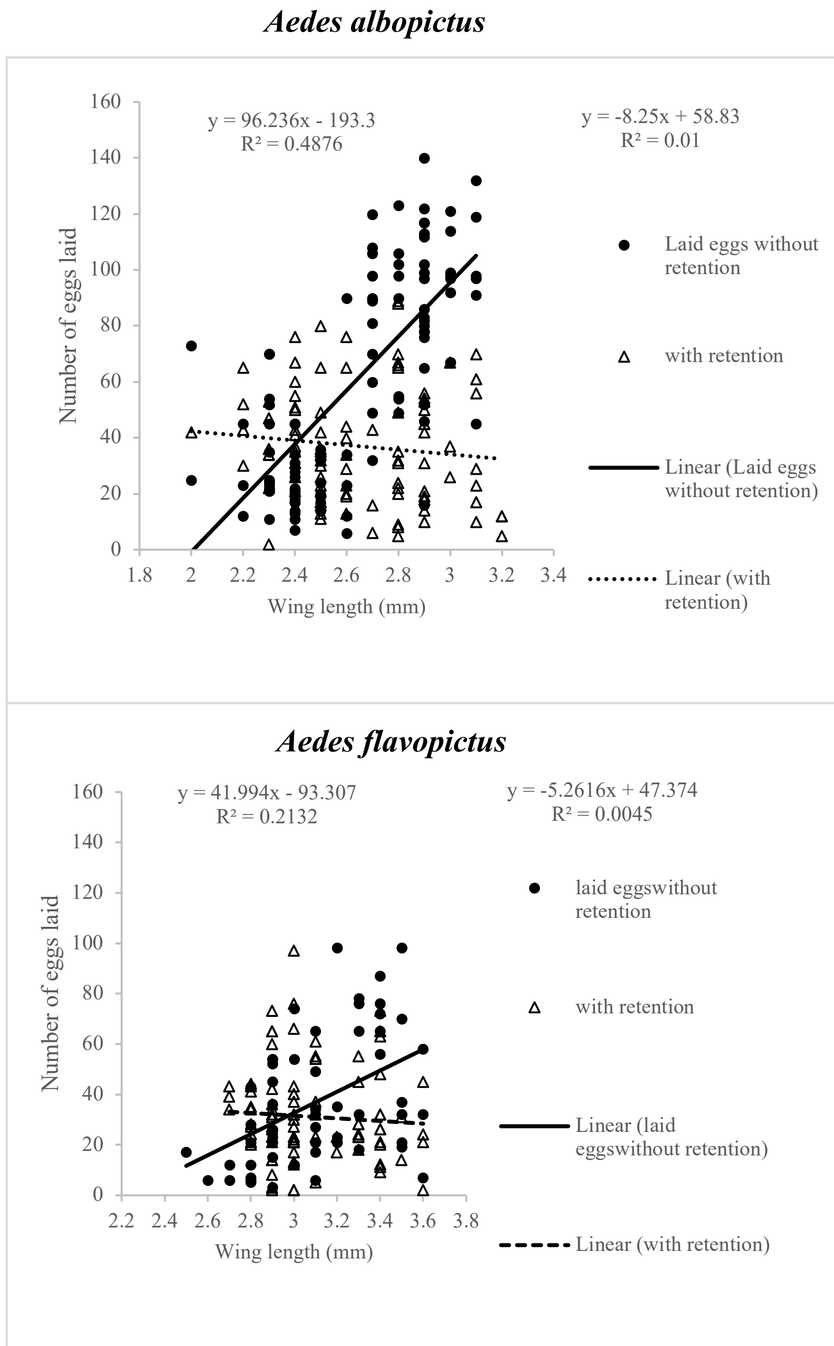


Fig. 5. Relationship between body size (wing length) and number of eggs laid (retention and without retention) in *Ae. albopictus* and *Ae. flavopictus*.

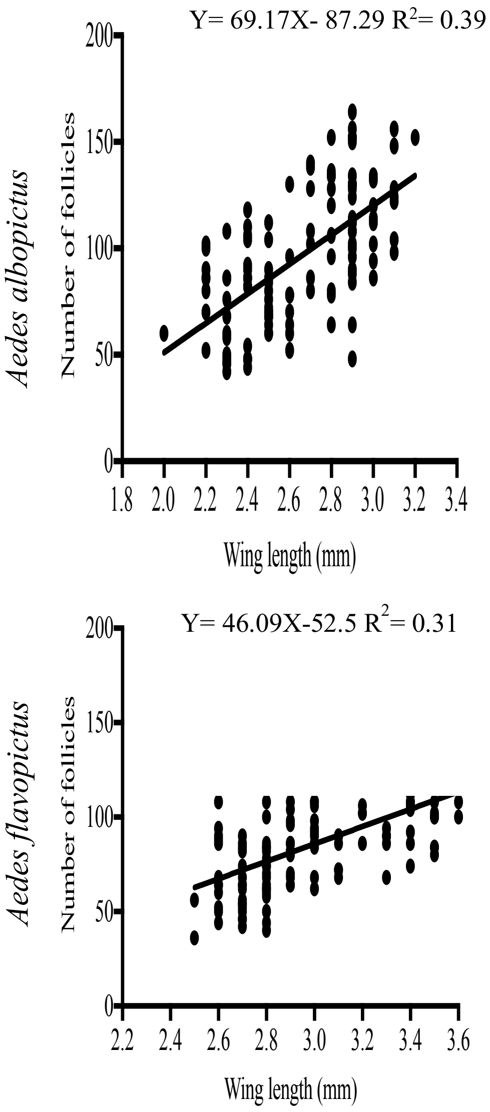


Fig. 6. The relationship between body size (wing length) and number of follicles in *Ae. albopictus* and *Ae. flavopictus*.

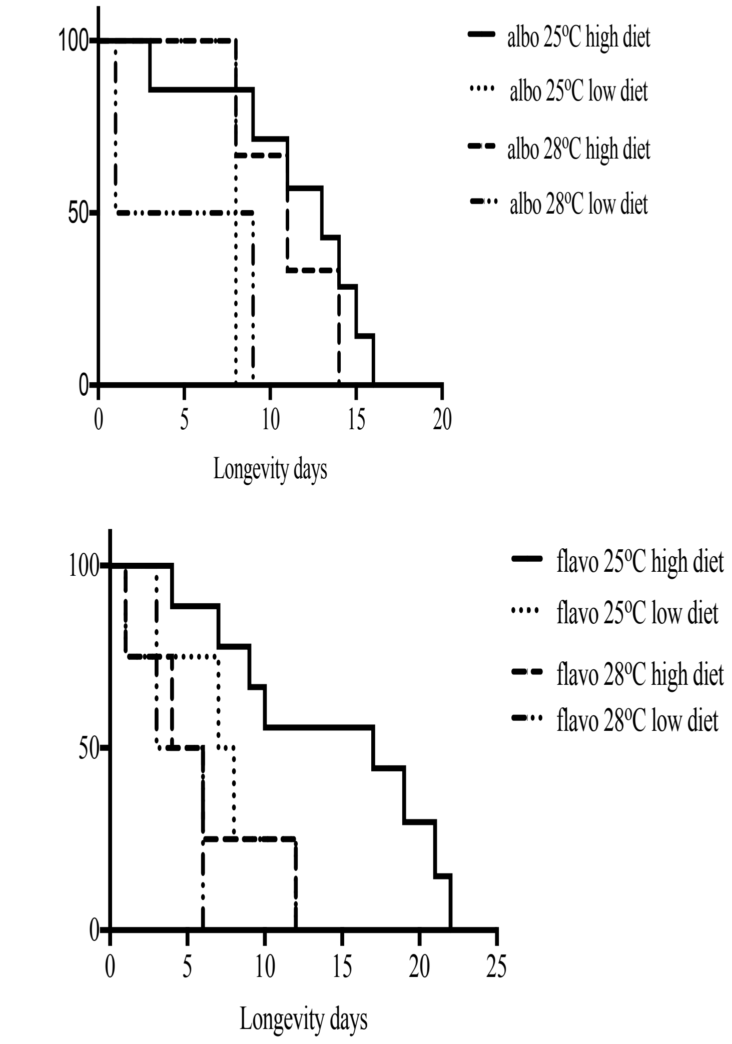


Fig. 7. Kaplan-Meier plots of the longevity (days) of *Ae. albopictus* and *Ae. flavopictus* at different temperature (25°C and 28°C) and diet (high and low).

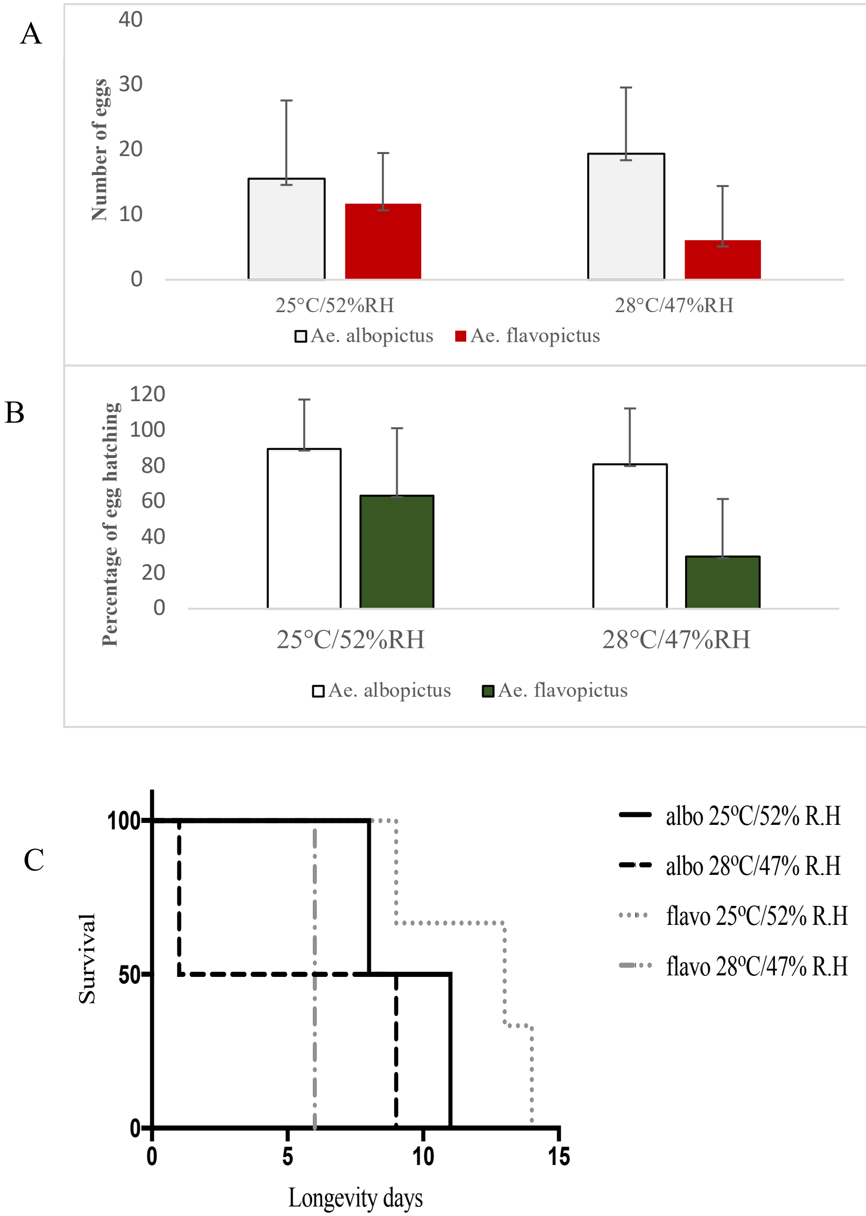


Fig. 8. Number of eggs (A), egg hatching (B) and longevity (C) of *Ae. albopictus* and *Ae. flavopictus* at different temperature and humidity.

令和 2 年8月3日

学位論文審査報告書（甲）

1．学位論文題目（外国語の場合は和訳を付けること。）

Studies of coexistence mechanism between *Aedes albopictus* and *Aedes flavopictus*

ヒトスジシマカとヤマダシマカの2種の共存機構の研究

2．論文提出者　(1) 所　　属　自然システム学　専攻

(2) 氏　　名　Sultana Afroza　スルタナ　アフロザ

3．審査結果の要旨（650字）

世界各地でアルボウイルス感染症媒介動物として問題となっている *Aedes albopictus* ヒトスジシマカは、アジアを起源とし、わずか 30 年で世界中に分布を拡大した。新天地で在来種を生殖干渉により排除するなど生物多様性にも影響している。生殖干渉とは近縁種の存在が繁殖を通し負の影響を与える現象である。ヒトスジシマカの属す *Stegomyia* 亜属の近縁種のうち琉球列島から北海道まで *Aedes flavopictus* ヤマダシマカが分布するため、ヒトスジシマカとも同所的に生息する。2種が共存するのはなぜか明らかにするため、生殖干渉について調査した。野外で採集されるヤマダシマカの体サイズが相対的に大きいことから体サイズが及ぼす影響を考慮し、雌雄の体サイズに変異のある組み合わせを作り交雑実験を行った。体サイズの大きいヒトスジシマカのメスは異種のオスを判別していたが、ヤマダシマカのメスは異種のオスを判別していないと考えられた。メカニズムは異なるが2種共に体サイズの大きいメスほど交雑による負の影響が少なかった。これまで石川県内で 2 種の rDNA の ITS 領域と mtDNA の COI 領域を調べた結果から2種間には遺伝子浸透が起こっていた。西南日本では2種の交尾前隔離が報告されていることと，我々の結果は異なる。温暖化に伴いヒトスジシマカの分布域は北上しており，もともと生息するヤマダシマカとの遭遇の歴史は北上するほど短い。2種の共存は、短期的には体サイズの大きいヤマダシマカが排除されにくく，長期的には2種共に交尾前隔離が進化することで成立すると考えられた。以上の研究結果は理学的に興味深く学位を与えるに値すると判断した。

4．審査結果　(1) 判　　定（いずれかに○印）　○合　格　・　不合格

(2) 授与学位　博　士（学術）