SUSCEPTIBILITY OF FIVE SPECIES MEMBERS OF THE KOREAN HYRCANUS GROUP TO *BRUGIA MALAYI*, AND HYBRIDIZATION BETWEEN *B. MALAYI*-SUSCEPTIBLE AND -REFRACTORY *ANOPHELES SINENSIS* STRAINS

Atiporn Saeung[1], Gi-Sik Min[2], Sorawat Thongsahuan[3], Kritsana Taai[1], Siripan Songsawatkiat[1] and Wej Choochote[1]

[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; [2]Department of Biological Sciences, Inha University, Incheon, South Korea; [3]Faculty of Veterinary Science (Establishment Project), Prince of Songkla University, Songkhla, Thailand

Abstract. Five species members of the Korean Hyrcanus Group: *Anopheles pullus*, *Anopheles sinensis*, *Anopheles kleini*, *Anopheles belenrae*, and *Anopheles lesteri* were tested for susceptibility to *Brugia malayi*. They were allowed to feed artificially on blood containing *B. malayi* microfilariae and dissected 14 days after feeding. The susceptibility rates were 60%, 65%, 90%, 100% and 100% in *An. pullus*, *An. sinensis*, *An. kleini*, *An. belenrae*, and *An. lesteri*, respectively. As determined by levels of susceptibility, results indicated that *An. pullus* was a moderate potential vector, while *An. sinensis*, *An. kleini*, *An. belenrae,* and *An. lesteri* were high potential vectors, when compared with the 90-95% susceptibility rates of an efficient control vector, *Ochlerotatus* (=*Aedes*) *togoi*. An introgressive study of *B. malayi*-susceptible/-refractory genes was performed intensively by hybridization experiments between a high (Korean strain) and a low (Thailand strain) potential *An. sinensis* vectors. The susceptibility rates of F_1-hybrids and backcross progenies were compared with parental stocks. The results indicated that the *B. malayi*-susceptible genes could be introgressed from a high to low potential *An. sinensis* vector by increasing the susceptibility rates from 0-5% in the parental stocks to 55% and 70% in F_1-hybrids and backcross progenies, respectively. The increase of susceptibility rates related clearly to the increase of normal larval development in the thoracic muscles of F_1-hybrids and backcross progenies.

Keywords: *Anopheles, Brugia malayi*, hybridization, Hyrcanus Group, susceptible/refractory genes, susceptibility level, Korea

INTRODUCTION

Anophelines of the Hyrcanus Group comprises at least 26 species members and have a wide distribution, extending from Iberia in Europe to East and Southeast Asian regions, including some of the off-lying islands of the Indian and Pacific oceans (Harrison and Scanlon, 1975; Tanaka *et al*, 1979; Harbach, 2012). It is recognized that some species members of the Hyrcanus Group are involved in the transmission of human diseases: ma-

Correspondence: Dr Atiporn Saeung, Department of Parasitology, Faculty of Medicine, Chiang Mai, University, Chiang Mai 50200, Thailand.
E-mail: atiporn44@yahoo.com

laria (*Plasmodium vivax*), filariasis (*Brugia malayi*), and Japanese encephalitis virus, particularly in the Oriental Region and contiguous parts of the eastern Palaearctic Region (Sasa, 1976; Zhang, 1990; Ree *et al*, 2001; Kanojia *et al*, 2003; Lee *et al*, 2007; Rueda *et al*, 2010; Joshi *et al*, 2011).

In the Republic of Korea (ROK), at least six species members (*Anopheles belenrae*, *An. kleini*, *An. lesteri*, *An. pullus*, *An. sinensis* and *An. sineroides*) of the Hyrcanus Group have been recognized (Tanaka *et al*, 1979; Rueda, 2005). Among these, *An. sinensis* was incriminated as a natural vector of lymphatic filariasis due to *B. malayi* in mainland ROK; whereas, *An. sinensis* and *An. lesteri* were reported as natural vectors of this filarial parasite in China (Sasa, 1976). Regarding control measures in the ROK, the reduction of microfilariae in the peripheral blood of carriers interrupts the mosquito-transmitted cycle by using mass, combined with selective, treatments with a microfilaricide (diethylcarbamazine: DEC) to microfilaria positive persons.

These measures were started in 1964, together with substantial economic growth and improved living standards, including environmental and personal hygiene. This filarial control program brought about complete elimination of this lymphatic filariasis in 2007 (Cheun *et al*, 2009). Despite complete success of the program, re-emergence at any time of this endemic disease should be kept in mind, even in thoroughly controlled endemic regions, where the possible environmental factors favor suitable conditions for the transmission-cycle. This was reported recently in other mosquito-borne diseases, *eg*, re-emergence of malaria due to *P. vivax* in the ROK (Chai *et al*, 1994; Park *et al*, 2000; Shim and Shin, 2002).

Regarding the information mentioned above, details of the natural vectors of *B. malayi* have been documented in only *An. sinensis* among six species members of the Korean Hyrcanus Group. Therefore, this information clearly emphasizes lack of knowledge on the vector competence to *B. malayi* of these anopheline mosquitoes. However, the information could be used as a robust primary guideline for a field control approach, when suspecting any anopheline species of being a transmitting vector in endemic areas of Brugian filariasis. Hence, this study describes the susceptibility to *B. malayi* of five species members of the Korean Hyrcanus Group: *An. belenrae*, *An. kleini*, *An. lesteri*, *An. pullus* and *An. sinensis*.

In addition, this paper reported an introgressive study of *B. malayi*-susceptible/-refractory genes between high (Korean strain) and low (Thailand strain) potential *An. sinensis* vectors was performed by hybridization experiments and comparison of susceptibility levels of F_1-hybrids and backcross progenies with parental stocks.

MATERIALS AND METHODS

Mosquito species, wild-caught, fully engorged females of *An. belenrae*, *An. kleini*, *An. pullus* and *An. sinensis* were collected from Paju City, Gyeonggi-do Province, while *An. lesteri* was collected from So-Rae District, Incheon City, ROK. Species identification of wild-caught females followed standard illustrated keys (Tanaka *et al*, 1979; Rueda *et al*, 2005). Subsequently, morphological identification (using intact morphology of eggs, larvae, pupal skins, and adult females) and molecular investigation (Joshi *et al*, 2010) were performed in F_1-progenies of iso-female lines to guarantee the exact identification of species.

The laboratory colonies of the five anopheline species were then established by pooling five iso-female lines of each anopheline species, using the techniques described by Kim *et al* (2003). These colonies were used for studies on susceptibility to *B. malayi* throughout the experiments.

Regarding the introgressive study of *B. malayi*-susceptible/-refractory genes, the parental stocks of *An. sinensis* Korean strain [a high potential vector for *B. malayi* (results obtained from this study)], *An. sinensis* Thailand strain [a low potential vector for *B. malayi* (Saeung *et al*, 2013)], and their F_1-hybrids and backcross progenies were used. As for the control vector, autogenous *Ochlerotatus* (=*Aedes*) *togoi* (Chanthaburi Province, eastern Thailand strain) was selected as a proven efficient laboratory vector for a wide-range of genera and species of filarial nematodes, including *B. malayi* (Jumkum *et al*, 2003).

Filarial *B. malayi* originated from a 20-year-old woman, who was a resident of Narathiwat Province, southern Thailand. Domestic cats were later infected experimentally with the parasite, which was maintained at the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, from 1982 to 1986, when it was transferred to Mongolian jirds (*Meriones unguiculatus*) and then maintained at the animal house of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (Choochote *et al*, 1986).

Blood containing *B. malayi* microfilariae

Preparation of blood containing *B. malayi* microfilaria followed the details as described recently (Saeung *et al*, 2013). Briefly, the jirds were inoculated intraperitoneally for at least 3 months with infective larvae of *B. malayi* and anesthetized deeply with ethylene ether. The microfilariae were collected by injecting 3 ml of Hank's Balanced Salt Solution (HBSS, pH 7.2-7.4) into the peritoneal cavity before withdrawing by peritoneal washing. The 0.05 ml of peritoneal-washed-rich microfilariae was mixed with 5 ml of human-heparinized blood (10 units of heparin/ml of blood), taken from human volunteers who had signed the consent form. Then, the adjusted microfilarial density ranged from approximately 250 to 350 microfilariae (mf)/20 l by using the human-heparinized blood for artificial feeding all of all the mosquito species.

Infection of mosquitoes with *B. malayi* microfilariae

Five-day-old adult female *Oc. togoi*, *An. belenrae*, *An. kleini*, *An. lesteri*, *An. pullus* and *An. sinensis* fasted for 24 hours and then were allowed artificial feeding simultaneously on blood-containing *B. malayi* microfilariae (microfilarial density = 305 and 297 mf/20 l in Experiments I and II, respectively), using the techniques and apparatus previously described (Chomcharn *et al*, 1980).

Similarly, 5-day-old female *An. sinensis* Korean and Thailand strains, and their F_1-hybrids and backcross progenies fasted for 24 hours and then were allowed artificial feeding simultaneously on blood-containing *B. malayi* microfilariae (microfilarial density = 323 and 346 mf/20 l in Experiments I and II, respectively), using similar procedures as mentioned above. Fourteen days after feeding, all infected mosquitoes were dissected in normal saline solution and examined under a dissecting microscope. The number of mosquitoes with one or more infective stage larvae in any part of the body (head, thorax, or abdomen) was recorded.

Determination of the possible factors affecting the level of susceptibility

The thorax of infected *An. sinensis* Korean and Thailand strains, and their F_1-hybrids and backcross progenies were torn in a drop of normal saline solution and examined under a compound microscope 4 days after feeding. The first stage (L_1) larvae were counted and scored as normal L_1 larvae if alive with intact morphology. The larvae were scored as melanized L_1 larvae if they had evidence of a retained stage and melanotic encapsulation and scored as degenerated L_1 larvae if they demonstrated vacuolated internal organs without any evidence of melanotic encapsulation.

Ethical considerations

The Animal Ethics Committee of Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand approved the protocols. Authors have provided substantiation of ethical approval.

RESULTS

Details of the infective rates and parasite loads of *Oc. togoi*, *An. belenrae*, *An. kleini*, *An. lesteri*, *An. pullus*, and *An. sinensis* 14 days after feeding on blood containing *B. malayi* microfilariae are shown in Table 1. The 95% and 90% infective rates corresponded to an average of 16.47 and 13.06 infective (L_3) larvae per infected *Oc. togoi* in Experiments I and II, respectively, which indicated that all feeding experiments were under conditions of appropriate *B. malayi* microfilarial densities in infected blood. The infective rates and average number of L_3 larvae per infected mosquito of *An. pullus*, *An. belenrae*, and *An. lesteri* in Experiment I were 60%/8.50, 100%/8.85, and 100%/10.90, respectively. Those in *An. kleini* and *An. sinensis* in Experiment II were 90%/5.39, and 65%/4.23, respectively.

Comparative statistical analyses of the infective rates and average number of L_3 larvae per infected mosquito were carried out between *Oc. togoi* and five *An. hyrcanus* species. The results indicated that the infective rates differed significantly only between *Oc. togoi* and *An. pullus* ($p<0.05$), whereas the average number of L_3 larvae per infected mosquito did not differ significantly only between *Oc. togoi* and *An. lesteri* ($p>0.05$). Notably, all infective larvae that recovered from the two experimental feedings were very active and found to distribute in all regions of the head, thorax, and abdomen. Also, their behavior was similar, with more than 65% of infective larvae migrating from the thorax to the head and proboscis.

Details of the infective rates and parasite loads of parental, F_1-hybrids and backcross progenies of *An. sinensis* Korean and Thailand strains, 14 days after feeding on blood containing *B. malayi* microfilariae, are shown in Table 2. The 65% and 60% infective rates corresponded to an average of 3.62 and 4.33 L_3 larvae per infected *An. sinensis* Korean strain in Experiments I and II, respectively, which indicated that all feeding experiments were under conditions of suitable *B. malayi* microfilarial densities in infected blood. The infective rates and average number of L_3 larvae per infected mosquito of *An. sinensis* Korean strain, *An. sinensis* Thailand strain, and their 2 F_1-hybrids [(female *An. sinensis* Korean strain x male *An. sinensis* Thailand strain)F_1 and (female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain) F_1] in Experiment I were 65%/3.62, 5%/1, 65%/3.92, and 55%/5.27, respectively.

Comparative statistical analyses of the infective rates and average number of L_3 larvae per infected mosquito were

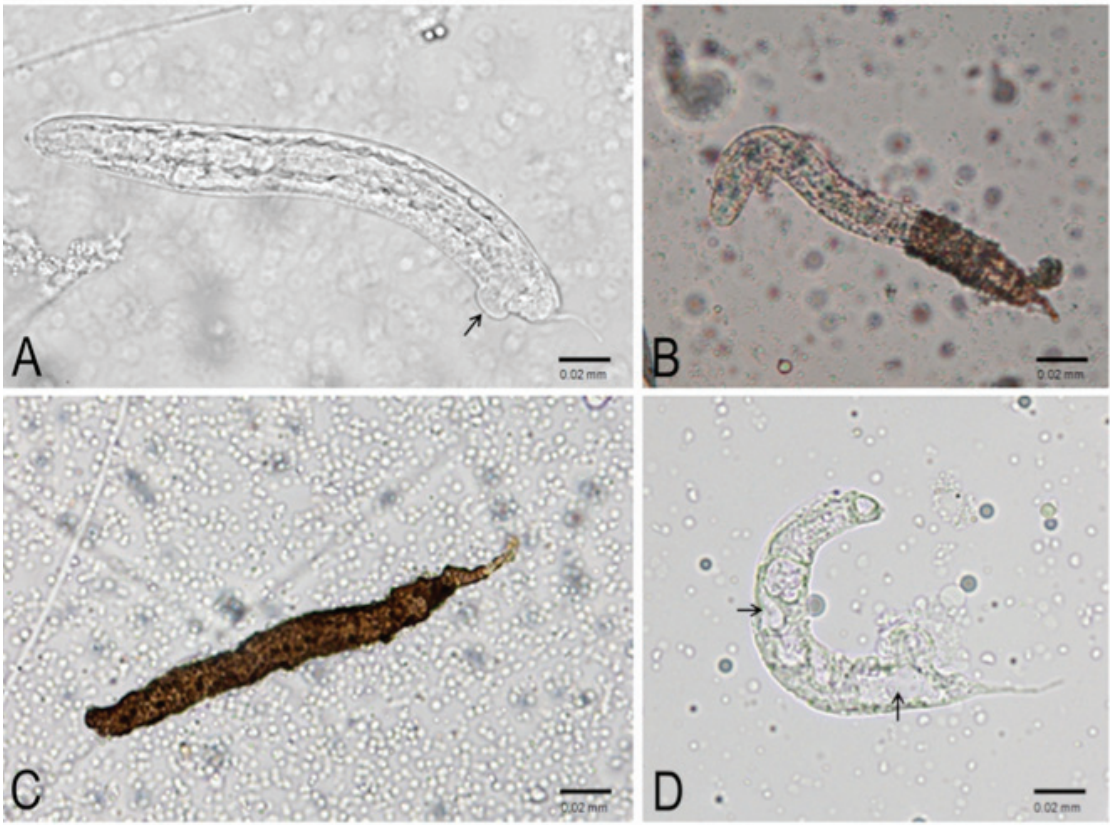

Fig 1–L_1 larvae recovered from thoracic muscle of *Anopheles sinensis* strains 4 days after infected blood meal. *An. sinensis* Korean strain: (A) Normal live larva with intact cuticle and internal organs (small arrow: protuberance of anal plug at the anal pore). *An. sinensis* Thailand strain: (B) Incomplete melanotic encapsulated larva. (C) Completely melanotic encapsulated larva. (D) Degenerated and vacuolated internal organs (small arrow) larva.

carried out between *An. sinensis* Korean strain and (female *An. sinensis* Korean strain x male *An. sinensis* Thailand strain) F_1, and *An. sinensis* Thailand strain and (female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain)F_1. The results indicated that the infective rates and average number of L_3 larvae per infected mosquito differed significantly only between *An. sinensis* Thailand strain and (female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain)F_1 ($p < 0.05$). The infective rates and average number of L_3 larvae per infected mosquito of *An. sinensis* Korean strain and *An. sinensis* Thailand strains, and their backcross progenies [(female *An. sinensis* Korean strain x male *An. sinensis* Thailand strain)F_1 x male *An. sinensis* Thailand strain], and [(female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain)F_1 x male *An. sinensis* Korean strain] in Experiment II, were 60% and 4.33, 0%, 45% and 4.22, and 70% and 5.50, respectively. Comparative statistical analyses of the infective rates and average number of L_3 larvae per infected mosquito were carried out between *An. sinensis* Korean strain and [(female *An.*

Table 1
Infective rates and parasite loads of five species in the Korean *Anopheles hyrcanus* group after feeding on blood containing *Brugia malayi* microfilariae (microfilarial density = 305 and 297 mf/20 l in Experiments I and II, respectively), with all mosquitoes dissected 14 days after feeding.

Mosquito species	Infective rate (%) (*n*)[a]	Average no. L_3 per infected mosquito (range)[b]	L_3-distribution		
			% head (*n*)	% thorax (*n*)	% abdomen (*n*)
Experiment I					
Oc. togoi	95 (19/20)	16.47 (1-37)	61.66 (193)	20.13 (63)	18.21 (57)
An. pullus	60 (12/20)a	8.50 (1-16)f	65.69 (67)	15.68 (16)	18.63 (19)
An. belenrae	100 (20/20)b	8.85 (1-21)g	75.71 (134)	15.25 (27)	9.04 (16)
An. lesteri	100 (20/20)c	10.90 (2-24)h	68.35 (149)	11.01 (24)	20.64 (45)
Experiment II					
Oc. togoi	90 (18/20)	13.06 (1-31)	66.81 (157)	14.89 (35)	18.30 (43)
An. kleini	90 (18/20)d	5.39 (1-10)i	76.29 (74)	11.34 (11)	12.37 (12)
An. sinensis	65 (13/20)e	4.23 (1-17)j	81.82 (45)	12.73 (7)	5.45 (3)

[a]Fisher's exact test: b, c, d, e *vs* control, $p > 0.05$; a *vs* control, $p < 0.05$.
[b]*t*-test (two-sided): h *vs* control, $p > 0.05$; f, g, i, j *vs* control, $p < 0.05$.

sinensis Korean strain x male *An. sinensis* Thailand strain)F_1 x male *An. sinensis* Thailand strain] and *An. sinensis* Thailand strain and [(female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain) F_1 x male *An. sinensis* Korean strain]. The results suggested that the infective rates and average number of L_3 larvae per infected mosquito differed significantly only between *An. sinensis* Thailand strain and [(female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain)F_1 x male *An. sinensis* Korean strain] ($p < 0.05$).

Parasite loads dissected 4 days after feeding on blood containing *B. malayi* microfilariae in parental, F_1-hybrids and backcross progenies of *An. sinensis* Korean and Thailand strains are detailed in Table 3 and Fig 1. A satisfactory average number of 19.40, 21.60, 23.20, and 18.20 L_1 larvae recovered from the thoracic muscles of *An. sinensis* Korean strain, *An. sinensis* Thailand strain, (female *An. sinensis* Korean strain x male *An. sinensis* Thailand strain) F_1, and (female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain)F_1, respectively, in Experiment I; and 24.60, 23.80, 20.40, and 25.60 L_1 larvae obtained from the thoracic muscles of *An. sinensis* Korean strain, *An. sinensis* Thailand strain, [(female *An. sinensis* Korean strain x male *An. sinensis* Thailand strain)F_1 x male *An. sinensis* Thailand strain], and [(female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain)F_1 x male *An. sinensis* Korean strain], respectively, in Experiment II, indicated that all of the mosquito species were successful in taking a considerable number of microfilariae from infected blood. Subsequently they invaded the cells of thoracic muscles.

However, low degrees of normal L_1 and high degrees of abnormal L_1 (melanized and degenerated L_1) larval development in the thoracic muscles of *An. sinensis* Thailand strain (normal L_1: 16.67-23.53%, abnormal L_1: 76.47-83.33%) clearly were different from those of *An. sinensis* Korean

Table 2
Infective rates and parasite loads in parental, reciprocal and backcross progenies of *Anopheles sinensis* strains from Korea and Thailand after feeding on blood containing *Brugia malayi* microfilariae (microfilarial density = 323 and 346 mf/20 l in Experiments I and II, respectively), with all mosquitoes dissected 14 days after feeding.

An. sinensis strains (Female x male)	Infective rates (*n*)[a]	Average *n* L_3 per infected mosquito (range)[b]	L_3-distribution		
			% head (*n*)	% thorax (*n*)	% abdomen (*n*)
Experiment I					
Parental crosses					
SK	65 (13/20)a	3.62 (1-13)e	72.34 (34)	10.64 (5)	17.02 (8)
ST	5 (1/20)b	1 (1)f	100 (1)	-	-
Reciprocal crosses					
(SK x ST)F1	65 (13/20)a	3.92 (1-16)e	80.39 (41)	11.76 (6)	7.84 (4)
(ST x SK)F1	55 (11/20)b	5.27 (1-16)f	48.27 (28)	31.03 (18)	20.70 (12)
Experiment II					
Parental crosses					
SK	60 (12/20)c	4.33 (1-11)g	88.46 (46)	9.62 (5)	1.92 (1)
ST	0 (0/20)d	-	-	-	-
Back crosses					
(SK x ST)F1 x ST	45 (9/20)c	4.22 (1-9)g	76.31 (29)	10.53 (4)	13.16 (5)
(ST x SK)F1 x SK	70 (14/20)d	5.50 (1-18)	64.94 (50)	18.18 (14)	16.88 (13)

SK, *An. sinensis* (Korean strain); ST, *An. sinensis* (Thailand strain).
[a]Chi-square test: a, c *vs* control, $p > 0.05$; b, d *vs* control, $p < 0.05$.
[b]*t*-test (two-sided): e, g *vs* control, $p > 0.05$; f *vs* control, $p < 0.05$.

strain (normal L_1: 48.45-56.10%, abnormal L_1: 43.90-51.55%), and their F_1-hybrids (normal L_1: 48.35-52.59%, abnormal L_1: 47.41-51.65%) and backcross progenies (normal L_1: 45.10-56.25%, abnormal L_1: 43.75-54.90%) of both directions.

DISCUSSION

To delineate a mosquito vector in an endemic area of filariasis, it is necessary to confirm the following evidence for a species of mosquitoes. Firstly, naturally caught specimens of a mosquito species contain infective stages of a parasite. Secondly, the same forms of infective stages develop in a laboratory-bred, clean colony of the same mosquito species after being fed on carrier blood containing parasites, and thirdly, the same mosquito species fed on human blood in an endemic area (Sasa, 1976). Therefore, from these criteria the susceptibility test in an experimental laboratory is a useful procedure for incriminating a potential vector of a certain species. Nevertheless, susceptibility alone does not imply an important role in the transmission of disease in nature, while a refractory one can rule out its significance entirely.

Vector competence to *B. malayi* of five species of the Korean *An. hyrcanus* group (*An. pullus*, *An. sinensis*, *An. kleini*, *An. belenrae*, and *An. lesteri*), as determined by susceptibility tests using a laboratory-bred, clean mosquito colony, had not been performed and/or reported until now. The results of this investigation indicated

Table 3
Parasite loads in parental, reciprocal and backcross progenies of *Anopheles sinensis* strains from Korea and Thailand dissected 4 days after feeding on blood containing *Brugia malayi* microfilariae (microfilarial density = 323 and 346 mf/20 l in Experiments I and II, respectively).

An. sinensis strains (Female x male)	Average *n* L_1 per infected thorax (range)[a]	% normal L_1 (*n*)	% melanized L_1 (*n*)	% degenerated L_1 (*n*)
Experiment I				
Parental crosses				
SK	19.40 (5-23)	48.45 (47)	24.74 (24)	26.81 (26)
ST	21.60 (8-31)	16.67 (18)	39.81 (43)	43.52 (47)
Reciprocal crosses				
(SK x ST)F1	23.20 (10-36)	52.59 (61)	22.41 (26)	25.00 (29)
(ST x SK)F1	18.20 (6-19)	48.35 (44)	21.98 (20)	29.67 (27)
Experiment II				
Parental crosses				
SK	24.60 (14-25)	56.10 (69)	26.83 (33)	17.07 (21)
ST	23.80 (9-44)	23.53 (28)	46.22 (55)	30.25 (36)
Back crosses				
(SK x ST)F1 x ST	20.40 (7-38)	45.10 (46)	30.39 (31)	24.51 (25)
(ST x SK)F1 x SK	25.60 (11-27)	56.25 (72)	21.09 (27)	22.66 (29)

[a]Dissected from five thoraxes.

that *An. sinensis*, *An. kleini*, *An. belenrae*, and *An. lesteri* were high potential vectors, whereas *An. pullus* was a moderate potential vector. Therefore, these present results confirm the natural vector status of *An. sinensis* in the ROK, and *An. sinensis* and *An. lesteri* in China (Sasa, 1976).

Beneficial results reported herein emphasize the potential role of *An. pullus*, *An. sinensis*, *An. kleini*, *An. belenrae*, and *An. lesteri* in transmitting *B. malayi* in the ROK, and *An. sinensis* and *An. lesteri* in China, where these anopheline species and *B. malayi* were found sympatrically. However, it is noteworthy that *An. sinensis*, *An. belenrae*, and *An. kleini* were cryptic morphologically, and only a molecular-based assay could be used robustly to recognize them (Rueda, 2005; Joshi *et al*, 2010). Remarkably, it is possible that previous identification of *An. sinensis* was based only on pure morphological characteristics, particularly in using traumatic scales of wild-caught adult females from endemic areas of Brugian filariasis, in which epidemiological and control approaches might be mixtures of two or three species, depending upon the locations studied.

It has been known that the f^m (filarial susceptibility, *B. malayi*) in *Aedes* species was controlled by simple sex-linked genes with refractoriness being dominant to susceptibility. The experiments of reciprocal and backcrosses between *B. malayi*-susceptible/-refractory strains of *Stegomyia* (=*Aedes*) *aegypti*, and *B. pahangi*-susceptible *Ae. polynesiensis*/-refractory *Ae. malayensis* produced refractory progeny-females, suggesting that refractoriness

is dominant to susceptibility (MacDonald and Ramachandran, 1965; MacDonald, 1976). However, those results are contrary to this study's experiments of reciprocal and backcrosses between *B. malayi*-susceptible (Korean strain)/-refractory (Thailand strain) *An. sinensis* by yielding susceptible progeny-females of both directions, indicating that susceptibility is dominant to refractoriness.

The decrease in melanized and degenerated of L_1 (2 main refractory mechanisms in the thoracic muscles of the Thai *An. sinensis*) (Saeung *et al*, 2013) from 39.81-46.22% melanization and 30.25-43.52% degeneration in parental *An. sinensis* (Thailand strain) to 21.98% melanization and 29.67% degeneration in F_1-hybrids (female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain) and 21.09% melanization and 22.66% degeneration in backcross progenies [(female *An. sinensis* Thailand strain x male *An. sinensis* Korean strain)F_1 x male *An. sinensis* Korean strain], when compared to 24.74-26.83% melanization and 17.07-26.81% degeneration of *An. sinensis* (Korean strain) were good supportive evidence. These results elucidated on a promising model of a *B. malayi*-anopheline-system for further investigations of various aspects concerning susceptibility/refractoriness mechanisms.

ACKNOWLEDGEMENTS

This work was supported by funding from the Thailand Research Fund (TRF Senior Research Scholar: RTA5480006) and Diamond Research Grant of the Faculty of Medicine, Chiang Mai University, awarded to W Choochote and A Saeung. The authors would like to thank Dr Wattana Navacharoen, Dean of the Faculty of Medicine, Chiang Mai University, for his interest in this research.

REFERENCES

Chai IH, Lim GI, Yoon SN, Oh WL, Kim SJ, Chai JY. Occurrence of tertian malaria in a male patient who never been abroad. *Korean J Parasitol* 1994; 32: 195-200.

Cheun HI, Kong Y, Cho SH, *et al*. Successful control of lymphatic filariasis in the Republic of Korea. *Korean J Parasitol* 2009; 47: 323-5.

Choochote W, Sukhavat K, Somboon P, Khamboonruang C, Maleewong W, Suwanpanit P. The susceptibility of small laboratory animals to nocturnally superiodic *Brugia malayi* in Thailand. *J Parasit Trop Med Assoc Thailand* 1986; 9: 35-7.

Chomcharn Y, Surathin K, Bunnag D, Sucharit S, Harinasuta T. Effects of a single dose of primaquine on a Thai strain of *Plasmodium falciparum*. *Southeast Asian J Trop Med Public Health* 1980; 11: 408-9.

Harrison BA, Scanlon JE. Medical entomology studies II. The subgenus *Anopheles* in Thailand (Diptera: Culicidae). *Contrib Am Entomol Inst* 1975; 12: 307 pp.

Harbach R. *Anopheles* classification. Mosquito taxonomic inventory. 2012 [Online]. [Cited 2013 May 10]. Available from: URL: http://mosquito-taxonomic-inventory.info/anophelesclassification%202013

Joshi D, Kim JY, Choochote W, Park MH, Min GS. Preliminary vivax malaria vector competence for three members of the *Anopheles hyrcanus* group in the Republic of Korea. *J Am Mosq Control Assoc* 2011; 27: 312-4.

Joshi D, Park MH, Saeung A, Choochote W, Min GS. Multiplex assay to identify Korean vectors of malaria. *Mol Ecol Resour* 2010; 10: 748-50.

Jumkum A, Choochote W, Jitpakdi A, *et al*. Comparative studies on the biology and filarial susceptibility of selected blood-feeding and autogenous *Aedes togoi* subcolonies. *Mem Inst Oswaldo Cruz* 2003; 98: 481-5.

Kanojia PC, Shetty PS, Geevarghese G. A long-term study on vector abundance

& seasonal prevalence in relation to the occurrence of Japanese encephalitis in Gorakhpur district, Uttar Pradesh. *Indian J Med Res* 2003; 117: 104-10.

Kim SJ, Choochote W, Jitpakdi A, Junkum A, Park SJ, Min GS. Establishment of a self-mating mosquito colony of *Anopheles sinensis* from Korea. *Korean J Entomol* 2003; 33: 267-71.

Lee WJ, Klein TA, Kim HC, *et al. Anopheles kleini*, *Anopheles pullus*, and *Anopheles sinensis*: potential vectors of *Plasmodium vivax* in the Republic of Korea. *J Med Entomol* 2007; 44: 1086-90.

MacDonald WW. Mosquito genetics in relation to filarial infections. In: Taylor AER, Muller R, eds. Genetic aspects of host-parasite relationship (Symposia of the British Society for Parasitology). Oxford, UK: Blackwell Scientific, 1976: 1-24.

MacDonald WW, Ramachandran CP. The influence of the gene f^{m} (Filarial susceptibility, *Brugia malayi*) on the susceptibility of *Aedes aegypti* to seven strains of *Brugia*, *Wuchereria* and *Dirofilaria*. *Ann Trop Med Parasitol* 1965; 59: 64-73.

Park JW, Son JI, Hur JP, *et al*. An outbreak of vivax malaria in Republic of Korea in 1999. *Korean J Infec Dis* 2000; 32: 335-9.

Ree HI, Hwang UW, Lee IY, Kim TE. Daily survival and human blood index of *Anopheles sinensis*, the vector species of malaria in Korea. *J Am Mosq Control Assoc* 2001; 17: 67-72.

Rueda LM, Li C, Kim HC, Klein TA, Foley DH, Wilkerson RC. *Anopheles belenrae*, a potential vector of *Plasmodium vivax* in the Republic of Korea. *J Am Mosq Control Assoc* 2010; 26: 430-2.

Rueda LM. Two new species of *Anopheles* (*Anopheles*) Hyrcanus Group (Diptera: Culicidae) from the Republic of Korea. *Zootaxa* 2005; 941: 1-26.

Sasa M. Human filariasis: a global survey of epidemiology and control. Tokyo: University of Tokyo Press, 1976: 433-67.

Saeung A, Hempolchom C, Baimai V, *et al.* Susceptibility of eight species members in the *Anopheles hyrcanus* group to nocturnally subperiodic *Brugia malayi*. *Parasit Vectors* 2013; 6: 5.

Shim JC, Shin EH. Malaria in Korea. *Korean J Infect Dis* 2002; 34: 104-35.

Tanaka K, Mizusawa K, Saugstad ES. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). *Contrib Am Entomol Inst* 1979; 16: 985 pp.

Zhang HL. [The natural infection rate of mosquitoes by Japanese encephalitis B virus in Yunnan Province]. *Zhonghua Yu Fang Yi Xue Za Zhi* 1990; 24: 265-7. [In Chinese with English abstract].