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Brief communication (Original)

Co-occurrence of mosquito larvae in stagnant water in residential areas in Malaysia

Van Lun Low[a], Chee Dhang Chen[a], Han Lim Lee[b], Phaik Eem Lim[a, c], Cherng Shii Leong[a], Mohd Sofian-Azirun[a]

[a]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, [b]Medical Entomology Unit, WHO Collaborating Centre for Vectors, Institute for Medical Research, Jalan Pahang, Kuala Lumpur 50588, [c]Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur 50603, Malaysia

Background: The importance of mosquito-borne diseases can be aggravated when there is an occurrence of mixed infestation between the mosquitoes in a habitat. However, there is limited available information on mixed infestation behavior among Malaysian mosquitoes.

Objective: We elucidated the nature of co-occurrence among mosquito species from residential areas in Malaysia.

Methods: Entomological investigation was carried out by using a previously described larval dipping method in 20 residential areas across 11 states and a federal territory (i.e., Kuala Lumpur) in Peninsular Malaysia as well as two states in East Malaysia.

Results: Of 20 study sites, eight study sites exhibited co-occurrence of mosquito larvae, ranging from 1.28% to 50.00%. *Culex quinquefasciatus* was able to breed simultaneously with *Cx. gelidus* (10.00%–50.00%), *Lutzia fuscanus* (2.94%–13.33%), *Cx. vishnui* (5.00%) and *Armigeres subalbatus* (1.28%–3.77%). On the other hand, *Cx. vishnui* was able to breed simultaneously with *Cx. gelidus* (20.00%) and *Lu. fuscanus* (3.33%).

Conclusion: The findings of this study have implications for the development of a better understanding of their mixed infestation behavior and prevention of vector-borne disease transmission from these study sites.

Keywords: *Armigeres*, *Culex*, *Lutzia*, co-occurrence, mixed infestation, Malaysia

To date, 442 species of mosquito representing 20 genera have been recorded in Malaysia [1]. Despite the importance of these mosquitoes in the potential for disease transmission, little is known about their mixed infestation behavior. In recent years, several studies have reported co-occurrence among *Aedes* larvae [2, 3] and co-occurrence between Anopheline and Culicine larvae [4]. However, no report has surfaced thus far pertaining to the mixed infestation behavior among *Culex* sp., *Lutzia* sp. and *Armigeres* sp. in stagnant water in residential areas in Malaysia.

The co-occurrence of more than one species in a habitat implies that they are sharing the same environmental conditions. However, different species of mosquitoes might spread different kinds of mosquito-borne diseases and certain diseases can be transmitted by more than one species of mosquito [1]. The importance of mosquito-borne diseases can be aggravated when there is an occurrence of mixed infestation between the mosquitoes in a habitat. It could be a serious problem in the attempt to assess their roles as vector-borne diseases during the outbreak of disease transmission. Besides, over-reliance of insecticide often causes resistant strains to evolve and different species of mosquitoes might have different rates of resistance development towards various classes of insecticides [5].

The present study focuses on the distribution and the incidence of co-occurrence among mosquito species from the residential areas in Malaysia. The findings of this study have implication for the development of a better understanding of their mixed infestation behavior and prevention of vector-borne disease transmission from these study sites.

Correspondence to: Van Lun Low, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia. E-mail: lucaslow24@gmail.com

Materials and methods

Entomological investigation was performed using a standardized larval dipping method in 20 residential areas in Malaysia. It has been confirmed that the surveillance period was free from the influence of northeast and northwest monsoon seasons. Mosquito larvae were dipped from stagnant water by using a 330 ml capacity dipper. Standardization of the number of dips in accordance with the surface area of the water body was conducted as follows: number of dips, water surface area (m^2): 1, < 0.25; 2, 0.26–1.0; 3, 1.1–3.0; 4, 3.1–5.0; 5, 5.1–7.0; 6, 7.1–9.0, and so on, as developed by Mendoza et al. [6]. Dips were taken gently with a 2–3 minute pause, to allow the mosquito larvae move freely in the air-water interface. Field-collected larvae were transported to the laboratory and were reared to adulthood for identification. Moribund and dead larvae were subsequently mounted for identification. The mosquito larvae and adults were identified according to taxonomic keys [7, 8].

Results

The percentage of co-occurrence of mosquito larvae obtained from larvae surveillance in Malaysia is demonstrated in **Table 1**. Eight study sites exhibited co-occurrence of mosquito larvae, namely Central Malacca (Malacca), Kota Bharu (Kelantan), Kuching (Sarawak), Ranau (Sabah), Senawang (Negeri Sembilan), Segamat (Johore), Shah Alam (Selangor) and Tuaran (Sabah).

The percentage of co-occurrence according to mosquito species is presented in **Table 2**. *Culex quinquefasciatus* was able to breed simultaneously with *Cx. gelidus* (10.00%–50.00%), *Lu. fuscanus* (2.94%–13.33%), *Cx. vishnui* (5.00%) and *Ar. subalbatus* (1.28%–3.77%). Meanwhile, *Cx. vishnui* was able to breed simultaneously with *Cx. gelidus* (20.00%) and *Lu. fuscanus* (3.33%).

The ratio of mosquito species recorded from co-occurrence dips is presented in **Table 3**. Generally, *Cx. quinquefasciatus* is the dominant species in the majority of dips conducted in Central Malacca (Malacca), Kota Bharu (Kelantan), Segamat (Johore) and Shah Alam (Selangor) by 1.50–10.00-fold. However, *Cx. vishnui* was the dominant species in dips conducted in Tuaran (Sabah) and Kuching (Sarawak) by 1.67–19.00-fold. It is of interest that the drains in Tuaran (Sabah) were inhabited by *Cx. vishnui*, *Cx. gelidus* and *Cx. quinquefasciatus* but the ratio of mixed infestation of these species were low (< 2).

Table 1. Percentage of co-occurrence of mosquito larvae in residential areas in Peninsular and East Malaysia

Study site	*Number of dip conducted	Positive dip		Co-occurrence found in positive dip	
		n	%	n	%
Kota Bharu, Kelantan	121	56	46.28	2	3.57
Kuala Terengganu, Terengganu	197	59	29.95	0	0.00
Kuantan, Pahang	167	19	11.38	0	0.00
Padang Besar, Perlis	123	11	8.94	0	0.00
Kuala Kedah, Kedah	144	17	11.81	0	0.00
Bayan Lepas, Penang	212	10	4.72	0	0.00
Sitiawan, Perak	196	81	41.33	0	0.00
Shah Alam, Selangor	187	34	18.18	1	2.94
Kepong, Kuala Lumpur	162	31	19.14	0	0.00
Senawang, Negeri Sembilan	331	15	4.53	2	13.33
Central Malacca, Malacca	131	53	40.46	2	3.77
Segamat, Johore	267	78	29.21	1	1.28
Kuching, Sarawak	218	30	13.76	1	3.33
Bau, Sarawak	86	5	5.81	0	0.00
Samarahan 1, Sarawak	103	10	9.71	0	0.00
Samarahan 2, Sarawak	113	3	2.65	0	0.00
Tuaran, Sabah	46	20	43.48	7	35.00
Likas, Sabah	162	8	4.94	0	0.00
Ranau, Sabah	53	2	3.77	1	50.00
Kota Kinabalu, Sabah	98	5	5.1	0	0.00
Total	**3117**	**547**	**17.55**	**17**	**3.11**

*Details on larval surveillance have been produced in our previous study [23].

Table 2. Percentage of co-occurrence according to mosquito species

Study site	Positive (n)	Dip (n)										
		CQ n (%)	**CV** n (%)	**CG** n (%)	**AS** n (%)	**LF** n (%)	**AN** n (%)	**CQ+LF** n (%)	**CQ+AS** n (%)	**CQ+CV** n (%)	**CQ+CG** n (%)	**CV+LF** n (%)	**CV+CG** n (%)
Kota Bharu, Kelantan	56	54 (96.43)	0	0	0	0	0	2 (3.57)	0	0	0	0	0
Shah Alam, Selangor	34	33 (97.06)	0	0	0	0	0	1 (2.94)	0	0	0	0	0
Senawang, Negeri Sembilan	15	13 (86.67)	0	0	0	0	0	2 (13.33)	0	0	0	0	0
Central Malacca, Malacca	53	51 (96.23)	0	0	0	0	0	0	2 (3.77)	0	0	0	0
Segamat, Johore	78	77 (98.72)	0	0	0	0	0	0	1 (1.28)	0	0	0	0
Kuching, Sarawak	30	14 (46.67)	15 (50.00)	0	0	0	0	0	0	0	0	1 (3.33)	0
Tuaran, Sabah	20	2 (10.00)	8 (40.00)	3 (15.00)	0	0	0	0	0	1 (5.00)	2 (10.00)	0	4 (20.00)
Ranau, Sabah	2	0	0	0	0	0	1 (50.00)	0	0	0	1 (50.00)	0	0
Total	**288**	**244 (84.71)**	**23 (7.99)**	**3 (1.04)**	**0**	**0**	**1 (0.35)**	**5 (1.74)**	**3 (1.04)**	**1 (0.35)**	**3 (1.04)**	**1 (0.35)**	**4 (1.39)**

CQ = *Cx. quinquefasciatus*, CV = *Cx. vishnui*, CG = *Cx. gelidus*, AS = *Ar. subalbatus*, LF = *Lu. fuscanus*, AN = *An.separatus*

Table 3. Ratio of mosquito species recorded from co-occurrence dips

Study site	CQ : LF	CQ : AS	CQ : CV	CQ : CG	CV : LF	CV : CG
Kota Bharu, Kelantan	3.33 : 1.00	0	0	0	0	0
Shah Alam, Selangor	10.00 : 1.00	0	0	0	0	0
Senawang, Negeri Sembilan	3.25 : 1.00	0	0	0	0	0
Central Malacca, Malacca	0	3.50 : 1.00	0	0	0	0
Segamat, Johore	0	1.50 : 1.00	0	0	0	0
Kuching, Sarawak	0	0	0	0	19.00 : 1.00	0
Tuaran, Sabah	0	0	1.00 : 1.67	1.22 : 1.00	0	1.00 : 1.51
Ranau, Sabah	0	0	0	1.00 : 1.25	0	0

CQ = *Cx. quinquefasciatus*, CV = *Cx. vishnui*, CG = *Cx. gelidus*, AS = *Ar. subalbatus*, LF = *Lu. fuscanus*

Discussion

The co-occurrence of mosquito species regardless of their distribution frequency might be caused by several factors. Interspecific competition between species was the obvious hypothesis tested and has been studied intensively [9-11]. However, several studies have failed to document clear evidence for interspecific competition [12, 13]. It has been suggested that mixed infestation between species might be caused by temporal and spatial variation, rapid and extensive urbanization, difference in fecundity between species, and difference in life-cycle duration between species [12, 14]. It is not surprising to note that *Cx. quinquefasciatus* was able to breed simultaneously with another four species of mosquito in this study as their co-occurrence with another mosquito species have been well-documented around the world. Mixed infestation between *Cx. quinquefasciatus* and *Aedes* mosquitoes has been reported from Malaysia [2] and Brazil [15]. Inversely, co-occurrence of *Cx. quinquefasciatus* with *Cx. nigripalpus* in Florida [16] and *Cx. dolosus affinis* in Brazil [15] has also been elucidated. In Kenya, *Cx. quinquefasciatus* also co-occur with *Anopheles gambiae* [17] and *An. arabiensis* [18]. In the present study, *Cx. vishnui* was found to be able to breed simultaneously with *Cx. quinquefasciatus*, *Cx. gelidus* and *Lu. fuscanus*. It has been reported that *Cx. vishnui* also co-occurs with *Cx. brevipalpis* and *Cx. vishnui* complex in India and Southeast Asia regions, respectively [19-20].

The finding of this study demonstrated that *Ar. subalbatus* only co-occurs with *Cx. quinquefasciatus*. However, previous study has pointed out that *Ar. subalbatus* was also able to breed simultaneously with a large group of mosquitoes (i.e., *Ae. krombeini*, *Ae. albolpictus*, *Cx. uniformis*, *An. elegans*, *Toxorhynchites splendens* and *Tripteroides aranoides*) in Sri Lanka [21].

Co-occurrence of *Lu. fuscanus* with *Cx. quinquefasciatus* and *Cx. vishnui* was recorded in the present study. A previous study found that this species acts as the predator when they co-occurred with *Ae. albopictus*, *An. sinensis*, *Cx. sitiens*, *Cx. quinquefasciatus*, and *Cx. vagans* in China [22]. However, the presence of *Lu. fuscanus*, which occurred in a very low frequency in the present study, did not seemed to be a predator of *Cx. quinquefasciatus* or *Cx. vishnui*.

Conclusion

Although we acknowledge that the present data is insufficient to interpret the mixed population of mosquitoes resulting from factors mentioned in the discussion; nevertheless, the present study has provided the first documented data on the co-occurrence of mosquito larvae among *Culex* sp., *Lutzia* sp. and *Armigeres* sp. in residential areas in Malaysia. The findings of this study indicate that preventive and control measures should be considered proactive when there is an occurrence of mixed infestation between the mosquito species. We certainly do not want to wait till the outbreak of disease transmission that might be spread by different species of mosquitoes. By then, it will be too late to instill remedial action. A more comprehensive study is needed and routine monitoring of vector-borne disease is indispensable in assisting local authorities to improve vector control strategies currently practiced in Malaysia.

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References

1. Miyagi I, Toma T. The mosquitoes of Southeast Asia. In: Ng FSP, Yong HS, editors. Mosquitoes and mosquitoe-borne diseases: biology, surveillance, control, personal and public protection measures. Kuala Lumpur: Academy of Science Malaysia; 2000.
2. Chen CD, Nazni WA, Lee HL, Seleena B, Mohd Masri S, Chiang YF, et al. Mixed breeding of *Aedes aegypti* (L.) and *Aedes albopictus* Skuse in four dengue endemic areas in Kuala Lumpur and Selangor, Malaysia. Trop Biomed. 2006; 23:224-7.
3. Wan-Norafikah O, Chen CD, Soh HN, Lee HL, Nazni WA, Sofian-Azirun M. Surveillance of *Aedes* mosquitoes in a university campus in Kuala Lumpur, Malaysia. Trop Biomed. 2009; 26:206-15.
4. Rohani A, Wan Najdah WMA, Zamree I, Azahari AH, Mohd NI, Rahimi H, et al. Habitat characterization and mapping of *Anopheles maculatus* (Theobald) mosquito larvae in malaria endemic areas in Kuala Lipis, Pahang, Malaysia. Southeast Asian J Trop Med Public Health. 2010; 41:821-30.
5. Hidayati H, Sofian-Azirun M, Nazni WA, Lee HL. Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae against malathion, permethrin and temephos. Trop Biomed. 2005; 22: 45-52.
6. Mendoza F, Ibanez-Bernal S, Cabrero-Sanudo FJ. A standardized sampling method to estimate mosquito richness and abundance for research and public health surveillance programmes. Bull Entomol Res. 2008; 98: 323-32.
7. Rattanarithikul R, Harbach RE, Harrison BA, Panthusiri P, Jones JW, Coleman RE. Illustrated keys to the mosquitoes of Thailand II. Genera *Culex* and *Lutzia*. Southeast Asian J Trop Med Public Health. 2005; 36: 1-97.
8. Rattanarithikul R, Harrison BA, Harbach RE, Panthusiri P, Coleman RE, Panthusiri P. Illustrated keys to the mosquitoes of Thailand. IV. *Anopheles*. Southeast Asian J Trop Med Public Health. 2006; 37:1-128.
9. Braks MAH, Hono□rio NA, Lounibos LP, Louren□o-De-Oliveira R, Juliano SA. Interspecific competition between two invasive species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. Ann Entomol Soc Am. 2004; 97: 130-39.
10. Costanzo KS, Mormann K, Juliano SA. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). J Med Entomol. 2005; 42:559-70.
11. Paaijmansa KP, Huijbena S, Githekoc AK, Takken W. Competitive interactions between larvae of the malaria mosquitoes *Anopheles arabiensis* and *Anopheles gambiae* under semi-field conditions in western Kenya. Acta Trop. 2009; 109:124-30.
12. Chan KL, Chan YC, Ho BC. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. Bull World Health Organ.1971; 44:643-9.
13. Reiskind MH, Wilson ML. Interspecific competition between larval *Culex restuans* Theobald and *Culex pipiens* L.(Diptera: Culicidae) in Michigan. J Med Entomol. 2008; 45:20-7.
14. Leisnham, PT, Juliano SA. Spatial and temporal patterns of coexistence between competing *Aedes* mosquitoes in urban Florida. Oecologia. 2009; 160: 343-52.
15. Tubaki RM, Menezes RMTD, Vesgueiro FT, Cardoso RP. Observations on *Haemagogus janthinomys* Dyar (Diptera: Culicidae) and other mosquito populations within tree holes in a gallery forest in the northwestern region of Sao Paulo State, Brazil. Neotrop Entomol. 2010; 39:664-70.
16. Hribar LJ. Larval habitats of potential mosquito vectors of West Nile virus in the Florida Keys. J Water Health. 2007; 5:97-100.
17. Muturi EJ, Mwangangi J, Shililu J, Muriu S, Jacob B, Kabiru E, et al. Mosquito species succession and physicochemical factors affecting their abundance in rice fields in Mwea, Kenya. J Med Entomol. 2007; 44: 336-44.
18. Muturi EJ, Mwangangi J, Shililu J, Jacob, BG, Mbogo C, Githure J, et al. Environmental factors associated with the distribution of *Anopheles arabiensis* and *Culex quinquefasciatus* in a rice agro-ecosystem in Mwea, Kenya. J Vec Ecol. 2008; 33:56-63.
19. Devi NP, Jauhari RK. Mosquito species associated within some western Himalayas phytogeographic zones in the Garhwal region of India. J Insect Sci. 2007; 7:1-10.
20. Sirivanakarn S. The systematics of *Culex vishnui* complex in Southeast Asia with the diagnosis of

three common species (Diptera: Culicidae). Mosq Sys. 1975; 7:69-85.
21. Amerasinghe FP. Observations on the mosquitoes (Dipera: Culicidae) of Udawattakele Forest, Sri Lanka. J Natn Sci Coun Sri Lanka. 1982; 10:81-97.
22. Jin L, Luo J, Fu Y, Xu S. Prey and feeding behavior of larval *Culex* (*Lutzia*) *fuscanus* (Diptera: Culicidae) in Shantou, Guangdong Province, China. J Med Entomol. 2006; 43:785-6.
23. Low VL, Chen CD, Lee HL, Lim PE, Leong CS, Sofian-Azirun M. Nationwide distribution of *Culex* mosquitoes and associated habitat characteristics at residential areas in Malaysia. J Am Mosq Control Assoc. 2012; 28:160-9.