Geographic distribution, host plants, and morphological variation of the currently radiating phytophagous ladybird beetle *Henosepilachna diekei*

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ABSTRACT

To overview the morphological and ecological diversification within a species of phytophagous ladybird beetle *Henosepilachna diekei*, information concerning its distribution and host plant use was studied. This species was found widely in South East and South Asia from the Philippines through the Greater Sunda islands, a western part of Lesser Sundas, the Malay Peninsula to Sri Lanka. *Mikania* species (Asteraceae), *Mikania micrantha* in particular, were the most frequently used host plants, but some other plants belonging to Lamiaceae or Acanthaceae were also used by some local populations. Each population is possibly monophagous. According to morphological analyses, seven populations from four host plants at six sites on four Indonesian islands were grouped into two, one occurring on Java and Kalimantan (Borneo) and another on Sulawesi and Lombok, indicating that they were separated by the Wallace line. This morphological distinction was not correlated with the host plant use.

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Introduction

Rapid lineage diversification in association with evolution of various ecological characters and differential niche utilization is often referred to as “adaptive radiation”, which is one of the major processes to generate biodiversity (Schluter 2000). This rapid lineage diversification is often coupled with morphological divergence between geographically, ecologically and phylogenetically distinct populations in single species (Gould and Woodruff 1990). As morphological diversification is often associated with species diversification through

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enhancing reproductive isolation such as lock-and-key (Sota & Nagata 2008; Sota & Tanabe 2010; reviewed in Masly 2012) or size assortative mating (Brown 1990; Jiang et al. 2013), the investigation of morphological variation other than ecological characters within currently radiating species is critical to understand the process of adaptive radiation.

Henosepilachna diekei Jadwiszczak & Węgrzynowicz (referred to as *Epilachna* sp. 3 in Katakura et al. 2001; see also Kobayashi et al. 2009; for the taxonomic treatment) is a phytophagous ladybird beetle that was described based on specimens from the Philippines (Dieke 1947). In our survey on phytophagous ladybird beetles in South East Asia, this species is also found from various localities in Indonesia and adjacent countries. In addition, there seem to be host races in this species. In Java, for example, there are two types of populations, one depending on *Leucas lavandulifolia* Sm. (Lamiaceae), and the other on the climbing hemp weed, *Mikania micrantha* Kunth (Asteraceae) (Katakura et al. 2001; Matsubayashi et al. 2011; Fujiyama et al. 2013). A series of field and laboratory experiments demonstrated that beetle populations from *L. lavandulifolia* do not grow on *M. micrantha* and those from *M. micrantha* do not grow on *L. lavandulifolia* (Matsubayashi et al. 2011; Nakano unpublished data). In addition, there is no reproductive isolation between these two types of populations except differential host plant use (Matsubayashi et al. 2011, 2013a, 2013b) suggesting that these two types are at the early stage of speciation driven by divergent host adaptation (i.e. “host race”, *sensu* Drès & Mallet 2002). We have further found that there are populations that show specialization to plants other than *M. micrantha* and *L. lavandulifolia* (Matsubayashi 2008). Phylogenetic analysis based on partial nucleotide sequences of the mitochondrial cytoplasmic oxidase subunit I (COI) gene revealed that the beetle populations from each host plant are not monophyletic (Fujiyama et al. 2013), indicating that the host shift could occur several times in different localities in Java. Katakura et al. (2001) further observed that the *H. diekei* populations from Sumatra, a neighbouring island of Java, use other host plants belonging to Lamiaceae. Hence, *H. diekei* provides a good example with which to investigate adaptive radiation under various geographic settings. Preliminary morphological observation indicated that there was a certain degree of morphological variation (body shape, elytral spot pattern, minute structure of male genitalia) between populations of *H. diekei* sampled on different host plants.

In this paper, we investigate the distribution and host plants of *H. diekei* in the Asian tropics and further analyse the morphological divergence between geographically and ecologically different populations. Although our preliminary morphological observation indicates some degrees of inter-population morphological variation, it is still unknown whether the morphological divergence is associated with differential host plant use or not. This kind of knowledge is essential to understand the pattern and process of morphological diversification during adaptive radiation.

Material and methods

Beetle distribution and host plants

We surveyed the occurrence and host plant use of *H. diekei* from 1980 to 2013 in almost all major islands of Indonesia (Java, Sumatra, Kalimantan, Sulawesi, Bali,

Lombok, Sumbawa, Flores, West Timor and Papua) and some other localities in South East Asia (the shaded areas in [Figure 2](#)). We recorded the absence or presence of the beetle, and if present, we collected the beetles and the host plants for morphological analysis or species identification. Species identification was made based on morphologies by K. Matsubayashi and H. Katakura for the beetles and by J. Yokoyama for the host plants.

Morphological analysis

Adult individuals collected were fixed in absolute alcohol and kept at -40°C . Six body characteristics (body length, pronotum length, pronotum width, elytral length, elytron width, elytral height; see [Figure 1A](#)) were measured using a Nikon SMZ1500 microscope (Nikon Instech, Co., Ltd.) by recording images with a Digital Sight camera system (DS-L1, Nikon Instech, Co., Ltd.). Image stacking for the dorsal and lateral views of habitus was performed with CombineZP (Hadley, 2010; <http://www.hadleyweb.pwp.blueyonder.co.uk/>). Then, males were dissected in distilled water, and genitalia were eviscerated, cleaned in alkali solution (0.1% KOH) for 12 hours, and then washed in 0.1% lactic acid. The number of hairs on the penis guide of the tegmen and the structure of the apical edge of the penis were examined and recorded using the same systems described above (see [Figure 1B, C](#)). According to a preliminary observation, the structure of the apical edge of the penis was classified into three types; emarginate (Type I), truncate (Type II) and convex (Type III) (see [Figure 6](#)).

Principal component analysis (PCA) was conducted with the six morphological characters using *princomp* functions in *R 2.15.0* software (R Development Core Team [2012](#)). After the principal component analysis, we squeezed the principal components based on the importance of components especially for cumulative proportion (in general, more than 80% is useful). Further, we detected the relative contribution of each morphological character for the selected principal components based on the factor loadings, showing correlation of the factor to the principal components. We then tested statistical differences in the value of selected morphological characters among beetle populations using one-way analysis of variance (i.e. *ao*v function) with subsequent multiple comparisons by Tukey HSD test.

Furthermore, we tested differences in the number of hairs on the penis guide and the types of apical edge of penis among populations using a Kruskal–Wallis test with multiple comparisons by *Kruskal.wallis* function in *R 2.15.0* software.

Results

Beetle distribution and host plants

Collection and observation records of *Henosepilachna diekei* and the host plant use were summarized ([Figure 2](#)).

Beetle distribution: *Henosepilachna diekei* was widely found from the southern part of the Malay Peninsula (Ban Han in Songkhla, Thailand and Kuala Lumpur in Malaysia) through the Greater Sunda Islands (Borneo, Sumatra, Java) to the western part of the Lesser Sunda Islands (Bali and Lombok) ([Figure 2](#)). This beetle was also

Figure 1. Morphological characters of *Henosepilachna diekei* measured in the present study. (A) Dorsal and lateral views of habitus with measured body parts; BL, body length; PL, pronotum length; PW, pronotum width; EL, elytra length; EW, elytron width; EH, elytra height. (B) Lateral view of tegmen (PA, paramera; H, hair on penis guide; PG, penis guide). (C) Lateral view of penis (P, Penis; PA, ventral view of apical edge of penis).

recorded from Gannoruwa, Sri Lanka (not shown in Figure 2). On the other hand, this species was not found in the eastern part of the Lesser Sundas (Flores, Sumbawa, West-Timor), Papua, and the southern part of Thailand (excluding the

Figure 2. Distribution and host-plant use of *Henosepilachna diekei* populations in South East Asia. The distribution of *H. diekei* was investigated in shaded islands/regions. Localities where the occurrence of *H. diekei* was observed were shown by the names and symbols for the host plants. Six beetle populations from five localities used for the morphological analysis were black-edged.

southernmost part) in the Malay Peninsula (Figure 2), despite the potential host plants of *H. diekei*, especially *Mikania micrantha*, being frequently observed in these areas (see below).

Host plants: *Henosepilachna diekei* used plants of at least three families and four genera in the area surveyed (Figure 2). Of these four genera, *Mikania* (Asteraceae) was the most widely used. The beetle populations using *M. micrantha* were observed widely on Java (see Fujiyama et al. 2013, for further information), Sumatra (Lampung, Padang and Lake Toba), Borneo (Balikpapan and Pontianak in Kalimantan, Kinabaru in Sabah), and Kuala Lumpur. In Kuching in Sarawak, in addition, feeding traces were found on this plant although no individual was collected. In Sulawesi, by contrast, the populations using *M. micrantha* were found in rather restricted areas of the southern lowland (Siwa and Tinokari), but not in the northern part (Manado and the vicinities). No population was from *M. micrantha* in Lombok. Some beetle populations also occurred on *Mikania* sp. aff. *cordata* (Burm. F.) B. L. Robinson at high altitudes (approximately > 600 m altitude) in Java (e.g. Mt Gede, Mt Halimun and Mt Salak in the western part), Bali and southern Sulawesi (Palopo). In Sri Lanka, *H. diekei* was collected on *Mikania scandens* B. L. Rob.

The following plants were used in restricted areas.

***Leucas lavandulifolia*:** Infestation of *H. diekei* was found only in west and central Java, although this plant was sporadically found in other regions, such as

west Sumatra (Padang), east Borneo (Balikpapan) and south Thailand (in the Malay Peninsula).

Leucas mollisima: Found only at Purwodadi Botanic Garden in East Java.

Leucas sp.: Found only at the foot of Mt Rinjani in Lombok.

Plectranthus spp. (this genus was previously assigned as *Coleus*, Grayer et al. 2010): In Sulawesi, *H. diekei* was found infesting wild and cultivated *Plectranthus* plants in the northern and southern parts such as Manado, Palopo and Rantepao (Figure 2). In other regions, *Plectranthus* plants were also commonly observed, but *H. diekei* was not observed on this plant.

Dicliptera sp.: Infestation by *H. diekei* only at Patuha, West Java (Figure 2).

Morphological analysis

Six body-size-related characters were measured for a total of 67 females and 67 males from seven populations at six localities in four major islands of Indonesia (see Figure 2): three populations from *Mikania micrantha* (Bogor, Balikpapan and Tinokari), two from *Leucas* spp. (Bogor and Lombok), and one population from each of *Dicliptera* sp. (Patuha) and *Plectranthus* spp. (Rantepao). Of these, two populations from Bogor, West Java, one from *Mikania* and the other from *Leucas* occurred side by side; i.e. they were sympatric. All characters were highly variable among and within populations (Table 1). In the principal component analysis, PC1 occupied 64.7% of the total variance, and PC2 occupied 14% (Table 2). Body and elytra length showed high factor loadings to PC1, and elytra height showed a high loading to PC2 (Table 2). The seven populations were not clearly discriminated by PC1 and PC2 (see Supplementary material, Fig. S1). Hence, we simply compared body length and elytral height across populations for the representatives of PC1 and PC2, respectively (Figure 3).

Body length was significantly variable among populations in females ($df = 6$, $F = 2.624$, $P = 0.025$) but not in males ($df = 6$, $F = 0.837$, $P = 0.546$) (Figure 4). Females of the Balikpapan population had much smaller bodies than other populations. On the other hand, elytra height was variable among populations both in females ($df = 6$, $F = 5.589$, $P < 0.001$) and in males ($df = 6$, $F = 7.462$, $P < 0.001$) (Figure 5), whereas host use seemed to be unrelated to this morphological divergence (Table 3, Figures 4, 5). In summary, there was a certain morphological divergence among populations specifically in the elytra height for both sexes, but there was no clear morphological distinction among populations with respect to geographic or ecological divergence.

There were significant among-population differences in the number of hairs on the penis guide ($df = 6$, Kruskal–Wallis $\chi^2 = 38.127$, $P < 0.001$) and the types of apical edge of penis ($df = 6$, Kruskal–Wallis $\chi^2 = 54.121$, $P < 0.001$). Specifically for the latter, there seemed to be a geographic divergence; i.e. populations from Java and Kalimantan, and those from Sulawesi and Lombok. The former population group possessed the emarginate (Type I) structure more frequently, whereas the latter population group possessed the convex (Type III) structure more frequently (Figure 6). The intermediate, truncate (Type II) structure was found in both population groups (see Figure 6). Here

Table 1. Summary of morphological measurements of seven populations of *Henosepilachna diekei*.

Species	Locality	Population	Host	Sex	No	BL		PL		PW		EL		EW		EH	
						Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>H. diekei</i>	Java	Bogor	M	Female	10	6.40	0.36	1.79	0.13	2.83	0.22	5.37	0.29	2.59	0.12	2.79	0.23
				Male	10	5.88	0.22	1.71	0.18	2.75	0.10	5.06	0.22	2.43	0.12	2.55	0.20
		L	L	Female	10	6.32	0.20	1.89	0.18	2.86	0.09	5.34	0.27	2.43	0.06	2.73	0.11
				Male	10	5.75	0.24	1.65	0.12	2.71	0.13	4.82	0.22	2.29	0.11	2.43	0.14
	Kalimantan	Patuha	D	Female	10	6.54	0.27	1.87	0.11	2.76	0.10	5.42	0.28	2.47	0.37	2.50	0.25
				Male	10	5.88	0.60	1.75	0.14	2.57	0.16	4.78	0.45	2.20	0.15	2.29	0.22
Sulawesi	Lombok	Balikpapan	M	Female	10	6.03	0.31	1.74	0.35	2.76	0.09	5.06	0.30	2.38	0.11	2.66	0.19
				Male	9	5.89	0.23	1.26	0.08	3.05	1.00	4.96	0.19	2.38	0.12	2.65	0.12
		Rantepao	P	Female	10	6.38	0.40	1.87	0.13	2.92	0.17	5.45	0.39	2.62	0.18	2.90	0.28
				Male	10	5.95	0.23	1.81	0.10	2.78	0.11	4.99	0.29	2.46	0.14	2.74	0.18
	Lombok	Tinokari	M	Female	10	6.36	0.33	1.93	0.18	2.93	0.17	5.43	0.32	2.52	0.17	3.05	0.19
				Male	10	5.88	0.20	1.65	0.15	2.77	0.09	4.94	0.22	2.35	0.09	2.62	0.15
Plectranthus	Lombok	Lombok	L	Female	7	6.47	0.26	1.92	0.06	2.91	0.07	5.42	0.34	2.60	0.15	2.88	0.37
				Male	8	6.07	0.26	1.80	0.09	2.75	0.09	5.08	0.31	2.50	0.13	2.61	0.17

BL, body length; PL, pronotum length; PW, pronotum width; EL, elytra length; EW, elytra width; EH, elytra height. Host plants are denoted as M, *Mikania*; L, *Leucas*; D, *Dicliptera*; P, *Plectranthus*.

Table 2. Summary of principal component analysis using six morphological measurements in seven populations of *Henosepilachna diekei*.

Factor	Component	
Importance of components	PC1	PC2
Standard deviation	0.666	0.310
Proportion of variance	0.647	0.140
Cumulative proportion	0.647	0.787
Factor loadings		
Body length (BL)	0.683	0.159
Pronotum length (PL)	0.170	-
Pronotum width (PW)	0.184	-0.197
Elytra length (EL)	0.638	0.143
Elytra width (EW)	0.198	-0.139
Elytra height (EH)	0.155	-0.945

again, host utilization seemed unrelated to this morphological divergence (Table 3, Figures 5, 6).

Discussion

The present study showed that *Henosepilachna diekei* is distributed widely in South East and South Asia, ranging from the Philippines through the Greater Sunda Islands, the western part of the Lesser Sundas, the southern part of the Malay Peninsula, to Sri Lanka. It also appeared that this species uses various plants belonging to Asteraceae, Lamiaceae and Acanthaceae. At the population level, however, this species is monophagous, i.e. each population depends on a single or some closely-related plant species. In consequence, two different populations that depend on different host plants sometimes occur in the proximity. For example, we observed that two populations, one depending on *M. micrantha* and the other on *L. lavandulifolia*, occurred side by side in Bogor, West Java. Our laboratory and field-cage experiments revealed that each of these two populations has a very strict host preference to its original plant, *M. micrantha* or *L. lavandulifolia*, and there is no reproductive isolation between these two populations except differential host specialization (Fujiyama et al. 2001; Matsubayashi et al. 2011, 2013a, 2013b). In our preliminary studies (K. Matsubayashi et al. unpublished), it was shown that beetle populations collected from other plant species also showed very strict preference for the original plants Hence, *H. diekei* seems to be in the process of radiation in South East Asia in association with the host plant shift.

It is uncertain which plants the ancestral *H. dikei* population used. At least, *M. micrantha*, a most widely used plant, and *M. scandens*, a plant used in Sri Lanka, are not ancestral host plants, because they were recently introduced to South East Asia from South America (Kostermans et al. 1987; Whitten et al. 1996) or North America. On the other hand, the original distributions of other host plants, *Mikania* sp. aff. *cordata*, *Leucas*, *Dicliptera* and *Plectranthus* species are still unclear. The speciation and colonization histories of these host plants should be studied.

1. Bogor (M)

2. Bogor (L)

3. Patuha (D)

4. Balikpapan (M)

5. Rantepao (P)

6. Tinokari (M)

7. Lombok (L)

Figure 3. Variation of the elytral pattern and abdominal melanism of *Henosepilachna diekei*. Dorsal view (top) and lateral view (middle) of habitus and ventral view of abdomen (bottom) in male specimens collected in Java (1–3), Kalimantan (4), Sulawesi (5, 6) and Lombok (7). Localities of collection were shown upper of each picture, and the host plants are denoted in the parentheses as M; *Mikania*, L; *Leucas*, A; *Asystacea*, C; "*Coleus*". Scale bar = 1 mm.

Henosepilachna diekei exhibited geographic variation among populations in elytral height, number of hairs on the penis guide, and the types of apical edge of penis. According to the present morphological analysis, specifically for the types of

Figure 4. Body length of seven populations of *Henosepilachna diekei*. (A) Females; (B) males. The host plants were denoted in the parentheses as M; *Mikania*, L; *Leucas*, D; *Dicliptera*, P; *Plectranthus*. The different letter on the right shoulder of each box indicates significant difference ($P < 0.05$) after adjustment of P -value for multiple comparisons (NS, $P \geq 0.05$).

Figure 5. Elytra height of seven populations of *Henosepilachna diekei*. (A) Females; (B) males. The host plants were denoted in the parentheses as M, *Mikania*; L, *Leucas*; D, *Dicliptera*; P, *Plectranthus*. The different letter on the right shoulder of each box indicates significant difference ($P < 0.05$) after adjustment of P -value for multiple comparisons (NS, $P \geq 0.05$).

Table 3. Number of hairs on penis guide in seven populations of *Henosepilachna diekei*.

No. hair	Locality	Population	Host plant	N	Mean	Median	SD	Difference
<i>H. diekei</i>	Java	Bogor	M	10	6.80	6.50	3.43	ab
			L	10	3.10	3.00	3.14	ab
			D	10	1.70	2.00	1.34	a
	Kalimantan	Balikpapan	M	13	8.23	8.00	2.05	b
	Sulawesi	Rantepao	P	10	1.50	0.50	2.22	a
		Tinokari	M	10	1.30	1.50	1.06	a
	Lombok	Lombok	L	8	3.00	3.00	3.12	ab

Host plants are denoted as M, *Mikania*; L, *Leucas*; D, *Dicliptera*; P, *Plectranthus*. The significant differences are indicated as different letter ($P < 0.05$) after adjustment of p-value for multiple comparisons.

Figure 6. Variation in the structure of apical edge of penis in males of the seven populations of *Henosepilachna diekei*. Type I, emarginate (filled symbol); Type II, truncate (dark grey symbol); Type III, convex (light grey symbol). Solid line denotes the Wallace line. The number in each pie chart shows the number of specimen. The host plants were shown in the parentheses as M, *Mikania*; L, *Leucas*; D, *Dicliptera*; P, *Plectranthus*. The different letter on the right shoulder of each box indicates significant difference ($P < 0.05$) after adjustment of P -value for multiple comparisons.

apical edge of penis, these seven populations were grouped into two, one including four populations from Java and Kalimantan and the other including three populations from Sulawesi and Lombok (Figure 6). In addition, males from Bali (10 individuals), Padang in Sumatra (four individuals) and Sri Lanka (two individuals) possessed emarginate or truncate apical edge of penis, a characteristic of the former

group. Hence, it seems that the two population groups of *H. diekei* are separated by the Wallace line (c.f. Stelbrink et al. 2012; see Figure 6). In our preliminary molecular phylogenetic analysis, it has also been shown that *H. diekei* populations from the east side of this line and those from the west side form distinct clusters (Matsubayashi 2008; Matsubayashi et al. unpublished data). The Wallace line is assumed to act as a barrier for dispersal in *H. diekei* populations, as this line acts as a barrier for movements of large animals (Tänzler et al. 2014; Barley et al. 2015). On the other hand, this line seemed not to affect the evolution of host plant use since diversification in host utilization was commonly found in areas to both the east and west of the Wallace line (Figure 2). Further population sampling and detailed phylogenetic work are necessary to provide a comprehensive scheme for the evolutionary history of diversification in the morphological characters and host plant utilization in this beetle species.

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Disclosure statement

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