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CONTENT

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EXTENT OF USE OF THE NOVEL FABACEOUS HOST *CENTROSEMA MOLLE* BY *HENOSEPILACHNA VIGINTIOCTOPUNCTATA* (COLEOPTERA: COCCINELLIDAE) IN NUSA TENGGARA, INDONESIA

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ABSTRACT

The herbivorous ladybird beetle *Henosepilachna vigintioctopunctata* depends primarily on solanaceous plants. The utilisation of a novel fabaceous host, centro (*Centrosema molle*), by this beetle has been reported from several regions in Southeastern Asia, but details of the distribution and feeding habits of *H. vigintioctopunctata* populations on centro are largely unknown. Our study aimed to gather basic information on the utilisation of centro by *H. vigintioctopunctata* in the Nusa Tenggara region of southeastern Indonesia. Field surveys indicated that *H. vigintioctopunctata* did not yet utilize centro in the wild in this region. However, in feeding-choice experiments in the laboratory, all beetle populations tested from this region had some potential to utilize centro, i.e., showed some feeding acceptance of this plant. Based on these results, we discuss the current status of the Nusa Tenggara *H. vigintioctopunctata* populations in using centro as a host.

Key words: adult feeding preference, *Centrosema molle*, *Henosepilachna vigintioctopunctata*, host plant specificity, *Solanum torvum*

INTRODUCTION

The herbivorous ladybird beetle *Henosepilachna vigintioctopunctata* (Fabricius) (Coleoptera: Coccinellidae: Epilachninae) is widely distributed across Asia and Oceania (Richards 1983, Katakura *et al*. 1988, 2001, Jadwiszczak & Węgrzynowics 2003). This species depends primarily on solanaceous plants, including cultivated crops (Schaefer 1983, Shirai & Katakura 1999, Katakura *et al*. 2001). The most important hosts of *H. vigintioctopunctata* in Indonesia are the eggplant *Solanum melongena* L. and the shrubby weed *S. torvum* Sw. (Kalshoven *et al*. 1981, Katakura *et al*. 1988, 2001), although *H. vigintioctopunctata* populations feeding on the fabaceous weed centro (*Centrosema molle* Benth.; formerly referred to as *C. pubescens*; see Fantz 1996, Schultze-Kraft 2003) have been reported from West Java, Central Java, and West Sumatra in Indonesia, and peninsular Malaysia (Nishida *et al*. 1997, Shirai & Katakura 2000, Katakura *et al*. 2001). Since a phylogenetic analysis of twelve Asian epilachnine species based on mitochondrial DNA sequence data clearly indicates that solanaceous plants are the ancestral hosts of

† Deceased in January 2005

H. vigintioctopunctata and related species (Kobayashi *et al*. 1998), the utilisation of a fabaceous plant by *H. vigintioctopunctata* is a derived feeding trait.

Although centro originated in South and Central America, its wide use for plantation cover and/or pasture has made it one of the most widely distributed legume species in the humid tropics worldwide (Teitzel & Chen 1992). In Java, this plant has been used as green manure and for plantation cover since the 19th century (Teitzel & Chen 1992), and it is now a common weed in rural and suburban areas in West Java and West Sumatra (Nishida *et al*. 1997). Thus, *H. vigintioctopunctata* began to utilize centro in Indonesia sometime during the past 200 years. Nishida *et al*. (1997) reported that beetles collected on solanaceous hosts exclusively preferred *S. torvum* to centro, whereas beetles collected on centro did not show a clear preference. They also reported that beetles living on solanaceous plants had significantly larger body size than those living on centro. Nishida *et al*. (1997) and Bush & Butlin (2004) have suggested that the utilisation of centro by *H. vigintioctopunctata* represents an incipient phase in the divergence of host races, i.e., conspecific populations that are more or less reproductively isolated from one another by different host specificity alone under sympatric conditions (Drès & Mallet 2002). Details of the distribution and feeding habits of *H. vigintioctopunctata* populations on centro in Indonesia, however, were largely unknown except for West Java and West Sumatra (Nishida *et al*. 1997).

The present study focused on several islands in the Nusa Tenggara region in southeastern Indonesia, with the goal of gathering basic information on the utilisation of centro by *H. vigintioctopunctata* populations in this region. We investigated host-use patterns of *H. vigintioctopunctata* populations in the wild, and conducted laboratory experiments to elucidate the feeding preference of adult beetles for centro. These data are essential to understand the spatial pattern of evolutionary changes in the feeding habits of *H. vigintioctopunctata*. Based on our results, we discuss the current status of centro utilisation by *H. vigintioctopunctata* populations in the Nusa Tenggara region.

MATERIALS AND METHODS

Field surveys

We conducted fieldwork on Sumbawa, Flores, and Timor Islands (Fig. 1) to determine the frequency of utilisation of centro by *H. vigintioctopunctata*. Rainfall in this region is seasonal, with the rainy season generally begins in early December and continues until the end of March (Hamada *et al*. 2002). On Sumbawa Island, field surveys took place in the rainy season (20–21 March 2009), whereas on Flores Island and Timor Island surveys were undertaken in the dry season (4–6 November 2004 and 6–7 November 2004, respectively). Surveys on Sumbawa Island were carried out at 18 sites in Sumbawa and Dompu Regency. Surveys on Flores and Timor Islands were carried out at 13 sites in Ende Regency and at nine sites in Kupang and Timor Tengah Selatan Regency, respectively (for details of census site locations, see Table 1).

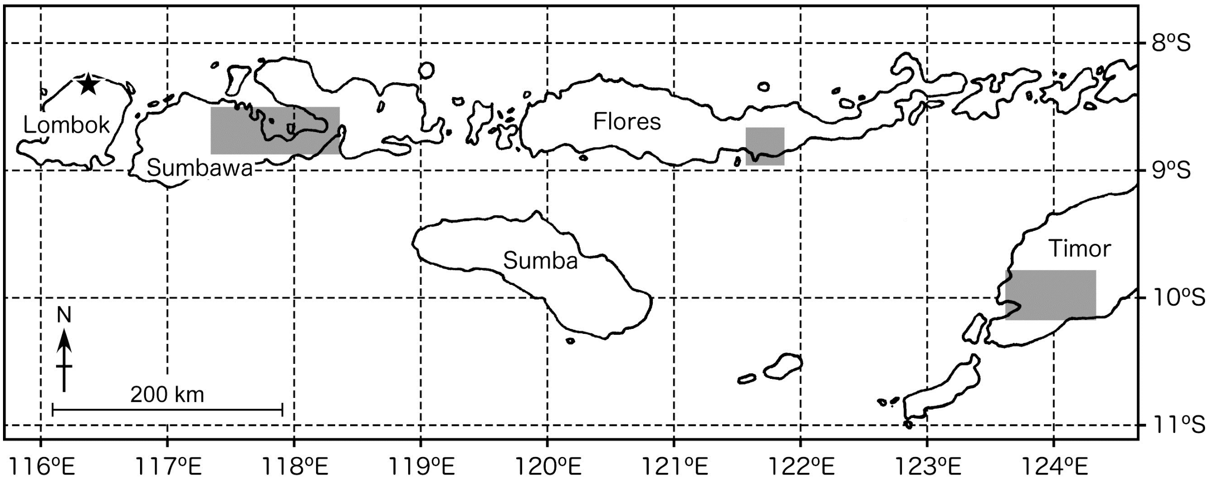

Figure 1. Map of the Nusa Tenggara region of Indonesia. Field surveys of host-use by *Henosepilachna vigintioctopunctata* were conducted in the shaded areas. The star indicates the collection site for individuals from the Lombok population used in laboratory experiments.

At each site, we checked for the presence of centro and solanaceous hosts. When we found host plants, we carefully checked for adult epilachnine beetles feeding on the leaves, and for epilachnine larvae and their characteristic lace-like feeding scars (Howard 1941). We collected beetles for use in laboratory experiments (see below), and for accurate identification.

Feeding choice experiments

We investigated the feeding preference for centro relative to *S. torvum* for adult *H. vigintioctopunctata* beetles collected during the field surveys, under uncontrolled ambient conditions at the Research Center for Biology-LIPI. In the feeding choice experiments, we examined as many beetle populations as possible for which 20 or more adults were collected on *S. torvum* at each census site. At Flores we also examined populations that occurred on *Brugmansia* sp. and/or for which fewer than 20 beetles were collected, because only one population matched the above criterion. At Timor Island, we found only one population of *H. vigintioctopunctata* occurring on *S. torvum* and *Brugmansia* sp. during field surveys (see Results), and we examined this population. In total, we examined five populations from Sumbawa, seven from Flores, and one from Timor (Fig. 2). Table 1 provides details of host use by each population in the wild.

We also examined one population from Lombok collected during another study by K.W. Matsubayashi *et al.* (unpublished data). Beetles from the Lombok population were collected on

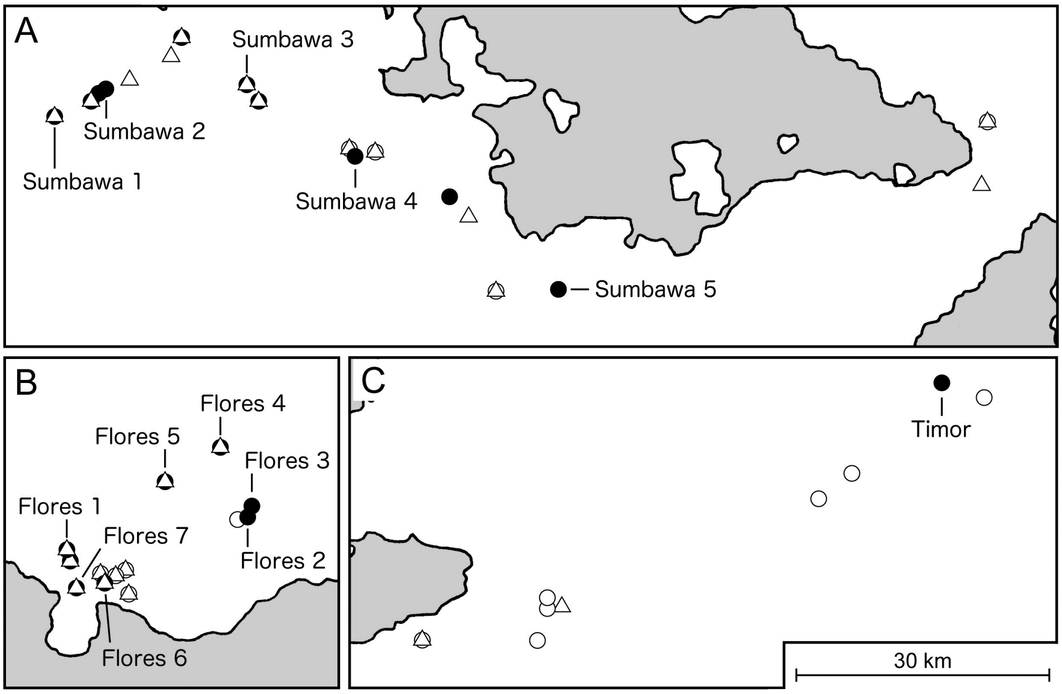

Figure 2. Sites at which host use by *Henosepilachna vigintioctopunctata* was examined: (A) Sumbawa, 2009; (B) Flores, 2004; (C) Timor, 2004. Circles, solanaceous hosts; triangles, centro. Filled symbols indicate the presence of both hosts and beetles, open symbols indicate hosts without beetles.

S. torvum, on 15 June 2008 at the foot of Mt. Rinjani, Lombok Utara Regency (8°18'06"S, 116° 24'27"E, 514 m a.s.l.; Fig. 1). Centro occurred at this site, but no epilachnine beetles were found on it.

In the feeding choice experiments, leaves of centro and *S. torvum* collected at Cibinong were cut to approximately the same area (12 cm^2) and placed in a transparent plastic case (6.0 x 5.5 x 2.0 cm) lined with moistened filter paper. A beetle was then released into the case and allowed to feed freely on the leaf pieces for 24 hours. Each beetle was tested twice, on each of two successive days. The leaves were then photocopied and scanned into a computer, and the leaf area consumed (cm^2) was measured with image processing software (ImageJ 1.45s; National Institutes of Health, Bethesda, Md., USA). The mean amount consumed per day was calculated for each beetle and host plant. For the Lombok population, each beetle was tested only once.

The results were analysed in two ways. First, a preference for either host was examined quantitatively with the *t*-test for paired comparisons, separately for each beetle population and sex. This statistical analysis was conducted in cases when 10 or more individuals had been tested for a beetle population and sex. Second, the qualitative feeding response to the two hosts, regardless of the amount consumed, was used to classify the beetles of each population and sex as either feeding on one of the hosts only, or feeding on both hosts. The frequency of individuals that accepted centro was then considered as an index of the potential of a population to utilize centro.

RESULTS

Field surveys

Results are summarised in Table 1. In the Sumbawa, Flores, and Timor areas, no epilachnine beetles were observed to feed on centro, either as adults or larvae. Outlines for the three areas are as follows.

Sumbawa (Fig. 2A, Table 1A). Solanaceous hosts and centro were observed at 14 and 13 of the 18 sites, respectively. Solanaceous plants and centro co-occurred at nine sites. Centro was often abundant and appeared to be of adequate condition to serve as a food resource for epilachnine beetles, as there were many young shoots with soft leaves. *Henosepilachna vigintioctopunctata* populations were found at 10 sites on solanaceous hosts (*S. torvum*, *S. erianthum* D. Don, *S. capsicoides* All., *Solanum* sp., *Brugmansia* sp., and *Datura metel* L.), including five sites where solanaceous hosts and centro co-occurred (Fig. 2A, Table 1A).

Flores (Fig. 2B, Table 1B). Solanaceous hosts were observed at all 13 sites, and centro was observed at 10 sites. Clumps of centro were usually small, and the density of this plant was low. *Henosepilachna vigintioctopunctata* populations were found at eight sites on solanaceous hosts (*S. torvum* and *Brugmansia* sp.), including six sites where solanaceous hosts and centro co-occurred. At one site where *H. vigintioctopunctata* occurred on *S. torvum* (Flores 1 population; Fig. 2B, Table 1B), centro was exceptionally abundant and sometimes twined around *S. torvum*. The census sites were still relatively dry when we conducted field surveys.

Timor (Fig. 2C, Table 1C). Solanaceous hosts were observed at eight and centro at two of the nine sites. Clumps of centro were moderate in size. Centro co-occurred with eggplant (*S. melongena*) at one site. A population of *H. vigintioctopunctata* was found at only one site, on *S. torvum* and *Brugmansia* sp. (the Timor population; Fig. 2C, Table 1C). The census sites were still relatively dry when we conducted field surveys.

Feeding choice experiments

Beetles of both sexes of all populations from Sumbawa, Flores, Timor, and Lombok consumed markedly larger amounts of *S. torvum* leaves than centro leaves at each population level (Table 2), and the feeding preference for *S. torvum* was always significant in cases statistically analyzed ($P \leq 0.001$ by *t*-test for paired comparisons).

For all populations investigated, no beetles fed only on centro leaves. In most populations from Sumbawa, Flores, and Timor, about 70% or more of individuals consumed small quantities of centro leaves (Table 2). The results for the Lombok population differed somewhat from those for populations from the other islands. Among 29 individuals examined, no females and only a small proportion of males consumed centro (Table 2C); one male (representing 3.4% of the individuals

Table 1. Results of field surveys to determine the frequency of utilisation of centro by *Henosepilachna vigintioctopunctata*

A) Sumbawa

Date	Locality name	Latitude	Longitude	Altitude (m)	Distribution of potential hosts[1] and utilisation by *H. vigintioctopunctata*	
					Solanaceous plants	Centro
20 March 2009	Batu Dulang, Batulanteh, Sumbawa	08°35'16"S	117°16'46"E	835	*Solanum torvum* with adults; the population Sumbawa 1 used in feeding experiments.	A small clump in quite good condition, not utilised.
	Klungkung, Batulanteh, Sumbawa	08°34'16"S	117°19'09"E	324	*Datura metel* and *Brugmansia* sp., both with adults.	A small clump, not utilised.
	—, Batulanteh, Sumbawa	08°33'41"S	117°19'36"E	374	*Solanum torvum* with adults and many trees of *S. erianthum* with adults and pupae.	(Not detected.)
		08°33'31"S	117°19'55"E	354	*Solanum torvum* with adults and pupae; the population Sumbawa 2.	(Not detected.)
	Semongkat, Unter Iwes, Sumbawa	08°32'49"S	117°21'32"E	310	(Not detected.)	A medium-sized clump on a fence, not utilised.
	—, Unter Iwes, Sumbawa	08°31'20"S	117°24'12"E	126	(Not detected.)	A large clump, not utilised.
		08°30'13"S	117°25'07"E	30	*Datura metel* with adults.	Occurring but not utilised.
21 March 2009	Karang Jati, Moyo Hilir, Sumbawa	08°33'12"S	117°29'19"E	28	*Solanum torvum* with adults; the population Sumbawa 3.	A large clump in good condition, not utilised.
	Serading, Moyo Hilir, Sumbawa	08°34'18"S	117°30'00"E	39	*Solanum capsicoides* and *Solanum* sp., both with adults.	A large clump in good condition, not utilised.
	Lape, Lape, Sumbawa	08°37'23"S	117°35'50"E	33	Cultivated *S. melongena*, not utilised.	A small clump, not utilised.
	Dete, Lape, Sumbawa	08°37'51"S	117°36'17"E	32	*Solanum torvum* with adults; the population Sumbawa 4.	(Not detected.)
	—, Lape, Sumbawa	08°37'31"S	117°37'34"E	24	Two small trees of *S. capsicoides*, not utilised.	A large clump in good condition, not utilised.
	Maronge, Maronge, Sumbawa	08°40'30"S	117°42'20"E	30	*Solanum capsicoides* and *Solanum* sp., both with adults and fourth-instar larvae.	(Not detected.)
	—, Maronge, Sumbawa	08°41'51"S	117°43'35"E	19	(Not detected.)	A medium-sized clump in good condition, not utilised.
	—, Plampang, Sumbawa	08°46'36"S	117°45'31"E	50	A small tree of *S. torvum* with feeding scars from unidentified epilachnine species.	A large clump in quite good condition, not utilised.
	Sepayung, Plampang, Sumbawa	08°46'29"S	117°49'33"E	3	A large tree of *S. torvum* with adults and pupae; the population Sumbawa 5.	(Not detected.)
	—, Manggelewa, Dompu	08°39'43"S	118°17'00"E	27	(Not detected.)	A medium-sized clump, not utilised.
	—, Manggelewa, Dompu	08°35'35"S	118°17'27"E	85	*Solanum capsicoides*, not utilised.	A small clump, not utilised.

[1] Information on plant abundance and leaf condition is not available, except in the cases mentioned.

Table 1 (continued)

B) Flores

Date	Locality name	Latitude	Longitude	Altitude (m)	Distribution of potential hosts[1)] and utilisation by *H. vigintioctopunctata*	
					Solanaceous plants	Centro
4 November 2004	Woloare, Ende Selatan, Ende	08°49'05"S	121°38'26"E	220	*Solanum torvum* with fourth-instar larvae.	A small clump, not utilised.
	Koanata, Ende Kelimutu, Ende	08°48'30"S	121°38'17"E	356	*Solanum torvum* with adults, pupae, larvae, and eggs; the popu- lation Flores 1 used in feeding experi- ments.	A large clump, not utilised. Sometimes twined around *S. torvum*.
5 November 2004	Moni, Ende Kelimutu, Ende	08°46'20"S	121°49'24"E	1394	*Solanum americanum* with *Henosepilachna* sp. adults other than *H. vigintioctopunctata*.	(Not detected.)
		08°46'14"S	121°50'01"E	1235	Several small trees of *Brugmansia* sp. with adults; the population Flores 2.	(Not detected.)
	Moni Waturaka, Ende Kelimutu, Ende	08°45'34"S	121°50'18"E	1030	*Brugmansia* sp. with adults; the population Flores 3.	(Not detected.)
	Nduaria, Ende Kelimutu, Ende	08°41'50"S	121°48'15"E	984	A small tree of *S. torvum* with adults; the population Flores 4.	Occurring sparsely, not utilised.
	Wolofeo, Detusoko, Ende	08°44'01"S	121°44'40"E	679	*Solanum torvum* with adults; the population Flores 5.	Occurring sparsely, not utilised.
6 November 2004	Manulondo, Ndona, Ende	08°49'38"S	121°42'02"E	249	Cultivated *S. melongena*, not utilised.	Occurring sparsely, not utilised.
	Onelako, Ndona, Ende	08°49'55"S	121°41'24"E	132	Cultivated *S. melongena*, not utilised..	Occurring sparsely, not utilised.
	Nanganesa, Ndona, Ende	08°50'36"S	121°40'48"E	20	*Brugmansia* sp. with adults; the population Flores 6.	Occurring sparsely, not utilised.
	Wolotopo, Ndona, Ende	08°51'08"S	121°42'09"E	15	*Solanum* sp., not utilised.	A small clump, not utilised.
	Wolowona, Ende, Ende	08°49'58"S	121°40'29"E	34	*Solanum torvum* with feeding scars from larvae of unidentified epilachnine species.	Occurring but not utilised.
	Mbongawani, Ende Selatan, Ende	08°50'54"S	121°38'54"E	17	Several trees of *S. torvum* with adults, pupae, and larvae; the population Flores 7.	Occurring sparsely, not utilised.

[1)] Information on plant abundance and leaf condition is not available, except in the cases mentioned.

Table 1 (continued)

C) Timor

Date	Locality name	Latitude	Longitude	Altitude (m)	Distribution of potential hosts[1)] and utilisation by *H. vigintioctopunctata*	
					Solanaceous plants	Centro
6 November 2004	Naibonat, Kupang Timur, Kupang	10°05'18"S	123°49'38"E	5	A small tree of *Solanum* sp., not utilised.	(Not detected.)
		10°06'02"S	123°49'35"E	0	Cultivated *S. melongena*, not utilised.	(Not detected.)
	Oesao, Kupang Timur, Kupang	10°08'07"S	123°48'59"E	19	Cultivated *S. melongena* with feeding scars from unidentified epilachnine species.	(Not detected.)
7 November 2004	Takari, Takari, Kupang	09°58'48"S	124°07'17"E	88	A tree of cultivated *S. melongena*, not utilised.	(Not detected.)
	Boentuka, Batu Putih, Timor Tengah Seratan	09°57'08"S	124°09'26"E	128	Field of *S. melongena* and three trees of *Solanum* sp., not utilised.	(Not detected.)
	Kesetnana, Mollo Selatan, Timor Tengah Seratan	09°51'23"S	124°15'16"E	857	Several trees of *Brugmansia* sp. and many trees of *S. torvum*, both with adults; the Timor population used in feeding experiments.	(Not detected.)
	Soe, Kota Soe, Timor Tengah Selatan	09°52'15"S	124°18'04"E	800	Five medium-sized trees of *S. torvum*, not utilised.	(Not detected.)
	Naibonat, Kupang Timur, Kupang	10°05'53"S	123°50'33"E	11	(Not detected.)	A large clump, not utilised.
	Tarus, Kupang Tengah, Kupang	10°08'02"S	123°41'27"E	10	Cultivated *S. melongena*, not utilised.	A medium-sized clump, not utilised.

[1)] Information on plant abundance and leaf condition is not available, except in the cases mentioned.

investigated for this population) was an exception, consuming 2.96 cm^2 of centro and 0.85 cm^2 of *S. torvum*.

When the results were compared between populations from different islands, populations from Sumbawa Island tended to consume larger amount of leaves than populations from Flores, Timor, and Lombok Islands (Table 2).

DISCUSSION

At Sumbawa and Flores, centro was commonly found and often grew in habitats where *H. vigintioctopunctata* occurred on solanaceous hosts (Fig. 2A, B; Table 1A, B). Even in cases where centro twined around the solanaceous plants utilised by *H. vigintioctopunctata*, however, no utilisation of centro by *H. vigintioctopunctata* was observed. On Timor, centro was scarce and was found at only two sites, although our surveys on this island were not extensive (Fig. 2C). Our results thus suggest that *H. vigintioctopunctata* has not begun to utilize centro on these islands. However, additional investigations at Flores and Timor during the rainy season are necessary, because dry weather seemed to result in centro of rather poor condition as a potential host for *H. vigintioctopunctata* on these islands in our census periods.

In the laboratory experiments, populations from Sumbawa Island tended to consume larger amount of leaves than those from Flores, Timor, and Lombok Islands (Table 2). This difference might be attributable to activity of beetles and/or the quality of leaves offered in the experiments, because only the Sumbawa populations were collected and examined during rainy season. In addition, the year of the experiments, host plants in the wild (*S. torvum* and/or *Brugmansia* sp.), and altitude of the habitat were often different between the beetle populations investigated (Tables 1, 2). All of these factors might affect the amount of leaves consumed by each beetle population.

Nevertheless, our laboratory experiments clearly indicated that all *H. vigintioctopunctata* populations examined from Lombok, Sumbawa, Flores, and Timor Islands showed some potential to use centro, i.e., showed some feeding acceptance of this plant (Table 2). In the Sumbawa, Flores, and Timor populations, a large fraction of individuals fed on centro as well as *S. torvum*, although the amounts of centro consumed were generally quite small (less than 0.25 cm^2 per individual; see also Table 2). Previously, Nishida *et al*. (1997) reported that beetles collected on solanaceous hosts exclusively preferred *S. torvum* to centro, whereas beetles collected on centro did not show a clear preference. The strong preference of *H. vigintioctopunctata* populations for solanaceous hosts (Table 2) (in other words, the absence of beetle individuals that utilised both solanaceous hosts and centro) would explain the lack of the utilisation of centro in Sumbawa, Flores, and Timor Islands. On the other hand, the Lombok population seemed to consist mainly of individuals with an

Table 2. Results of feeding choice experiments in which leaves of *Solanum torvum* and centro were simultaneously offered to individual adult *Henosepilachna vigintioctopunctata* beetles. See Figure 2 for locations of the populations listed

A) Sumbawa populations

Population	Host plant in the wild	Sex	*N*	Amount consumed in cm^2 (mean ± standard error) on:		% of individuals accepting centro
				S. torvum	Centro	
Sumbawa 1	*S. torvum*	Female	8[1)]	5.32 ± 0.388	0.05 ± 0.031	62.5
		Male	16	2.38 ± 0.141	0.05 ± 0.033	68.8
				Sexes pooled		66.7
Sumbawa 2	*S. torvum*	Female	13	6.02 ± 0.672	0.35 ± 0.164	84.6
		Male	10	3.75 ± 0.380	0.08 ± 0.033	70.0
				Sexes pooled		78.3
Sumbawa 3	*S. torvum*	Female	13	4.83 ± 0.222	0.06 ± 0.029	84.6
		Male	19	1.45 ± 0.202	0.02 ± 0.009	57.9
				Sexes pooled		68.8
Sumbawa 4	*S. torvum*	Female	8[1)]	4.40 ± 0.062	0.08 ± 0.068	62.5
		Male	13	1.58 ± 0.298	0.01 ± 0.006	76.9
				Sexes pooled		71.4
Sumbawa 5	*S. torvum*	Female	16	5.04 ± 0.323	0.17 ± 0.078	75.0
		Male	22	2.82 ± 0.230	0.12 ± 0.061	81.8
				Sexes pooled		78.9

[1)] No *t*-test for paired comparisons was conducted because of small sample size.

Table 2 (continued)

B) Flores populations

Population	Host plant in the wild	Sex	*N*	Amount consumed in cm^2 (mean ± standard error) on:		% of individuals accepting centro
				S. torvum	Centro	
Flores 1	*S. torvum*	Female	14	3.30 ± 0.204	0.28 ± 0.131	71.4
		Male	11	1.99 ± 0.223	0.05 ± 0.023	81.8
				Sexes pooled		76.0
Flores 2	*Brugmansia* sp.	Female	11	2.79 ± 0.412	0.24 ± 0.134	72.7
		Male	21	1.40 ± 0.132	0.18 ± 0.099	76.2
				Sexes pooled		75.0
Flores 3	*Brugmansia* sp.	Female	11	2.49 ± 0.378	0.17 ± 0.097	72.7
		Male	12	1.23 ± 0.129	0.23 ± 0.116	75.0
				Sexes pooled		73.9
Flores 4	*S. torvum*	Female	3[1)]	3.94 ± 0.605	0.01 ± 0.012	33.3
		Male	1[1)]	1.48	0.03	100.0
				Sexes pooled		50.0
Flores 5	*S. torvum*	Female	4[1)]	3.15 ± 0.684	0.04 ± 0.023	100.0
		Male	3[1)]	1.97 ± 0.323	<0.01 ± 0.002	33.3
				Sexes pooled		71.4
Flores 6	*Brugmansia* sp.	Female	11	3.01 ± 0.468	0.32 ± 0.103	90.9
		Male	7[1)]	1.91 ± 0.295	0.80 ± 0.285	71.4
				Sexes pooled		83.3
Flores 7	*S. torvum*	Female	7[1)]	2.16 ± 0.327	0.04 ± 0.014	71.4
		Male	4[1)]	1.07 ± 0.287	<0.01 ± 0.002	50.0
				Sexes pooled		63.6

[1)] No *t*-test for paired comparisons was conducted because of small sample size.

Table 2 (continued)

C) Timor and Lombok populations

Population	Host plant in the wild	Sex	*N*	Amount consumed in cm^2 (mean ± standard error) on:		% of individuals accepting centro
				S. torvum	Centro	
Timor	*S. torvum* and *Brugmansia* sp.	Female	17	2.79 ± 0.320	0.12 ± 0.045	76.5
		Male	14	1.00 ± 0.100	0.06 ± 0.030	78.6
				Sexes pooled		77.4
Lombok	*S. torvum*	Female	9[1)]	3.22 ± 0.453	0 ± 0	0
		Male	20	1.08 ± 0.182	0.15 ± 0.148	5.0
				Sexes pooled		3.4

[1)] No *t*-test for paired comparisons was conducted because of small sample size.

exclusive preference for solanaceous hosts, with a few exceptional individuals showing high acceptance of centro (Table 2C). Interestingly, no utilisation of centro was observed at the site where beetles of the Lombok population were collected, although centro occurred there.

Although utilisation of centro by *H. vigintioctopunctata* is common in West Java and West Sumatra (Nishida *et al*. 1997, Shirai & Katakura 2000, Katakura *et al*. 2001), the process of host-range expansion by this beetle remains unknown. The present study suggests that an incipient ability to feed on centro might be widespread in populations of *H. vigintioctopunctata* that at present do not utilise centro in nature. This ability might be inherent in *H. vigintioctopunctata* individuals before they encounter centro (see also Kikuta *et al*. 2013), or it is derived from western populations utilising centro. The ability of larvae to develop on centro is another important index for estimating the potential of beetle populations to use centro (Shirai & Katakura 1999, 2000). This aspect should be investigated in future studies.

Finally, we note that the utilisation of centro by *H. vigintioctopunctata* populations from the Nusa Tenggara region reported here may be highly specific to the times and places our investigations were carried out. Our ongoing studies in West Java and Bali have demonstrated that *H. vigintioctopunctata* can rapidly adapt to centro within several years, and inter-populational variation in the degree of this adaptation can occur on scales within a dozen kilometers (our unpublished data). For a better understanding of the spatiotemporal pattern of evolutionary changes in feeding habits in *H. vigintioctopunctata*, further investigations are needed from all over Indonesia. Furthermore, additional data from Nusa Tenggara could elucidate possible temporal changes in the area studied herein.

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