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CONTENT

POTENTIAL ABILITY OF THE *SOLANUM*-FEEDING LADYBIRD BEETLE *HENOSEPILACHNA DIFFINIS* (COLEOPTERA; COCCINELLIDAE) TO USE THE INTRODUCED FABACEOUS PLANT *CENTROSEMA MOLLE* IN EAST KALIMANTAN, INDONESIA

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ABSTRACT

Host specificity has been a major factor in generating the tremendous diversity of phytophagous arthropods. Studies of adaptation to introduced or invasive plant species provide an opportunity to investigate incipient evolutionary changes in host specificity. We investigated the cryptic ability of the Asian tropical herbivorous ladybird beetle *Henosepilachna diffinis* to feed on the fabaceous weed "centro", *Centrosema molle*, which was introduced to Southeast Asia about 200 years ago. In laboratory choice tests using this plant and the normal host plant, *Solanum torvum,* adults preferred *S. torvum* to centro, but over half the beetles tested ate leaves of both plants. Furthermore, most first-instar larvae accepted centro during a rearing experiment, and a few of them grew to the third-instar stage, though none reached the final (fourth) instar. *Henosepilachna diffinis* likely acquired this incomplete acceptability of centro without any direct host -grazer interaction with centro, probably before this weed was introduced to Southeast Asia. Our results further suggest that another *Henosepilachna* species*, H. vigintioctopunctata*, might similarly have already acquired an incomplete ability to use centro when this beetle encountered it for the first time, and this triggered a subsequent host-range expansion from solanaceous plants to include centro in various parts of Southeast Asia.

Key words: adult feeding preference, *Centrosema molle*, *Henosepilachna,* host plant specificity, solanaceous hosts

INTRODUCTION

All phytophagous arthropods are more-or-less restricted in the plant species they utilise (*e.g.*, Bernays & Chapman 1994). This host specificity is thought to have been a major factor in the tremendous radiation of phytophagous arthropods (*e.g.* Janz *et al*. 2006, Futuyma & Agrawal 2009). The adaptation of phytophagous arthropods to introduced or invasive plant species can provide an opportunity to investigate incipient evolutionary changes in host specificity, and dozens of empirical studies have dealt with host shifts or expansion by phytophagous arthropods to such plants (*e.g.*, Singer *et al*. 1993, Andow & Imura 1994, Feder 1998, Denno *et al.* 2008). For example, the nymphalid butterfly *Euphydryas editha* (Boisduval) rapidly adapted to an exotic host in North America; it began to utilise the Eurasian plant *Plantago lanceolata* L. (Plantaginaceae) at Carson, Nevada (USA) in less than 100 years after the plant's first arrival there (Singer *et al.* 2008).

In this case, genetic variation in female butterfly oviposition preference and the ability of the larvae to develop on the exotic host appear to have been present before the butterfly encountered the novel host species (Singer *et al.* 2008).

The fabaceous weed centro *Centrosema molle* Benth. (formerly referred to as *C. pubescens*; see Fantz 1996, Schultze-Kraft 2003) was introduced to Indonesia from South America in the 19th Century as a source of green manure, and it has since spread to most Indonesian islands and to other countries in Southeast Asia and Oceania (Teitzel & Chen 1992, N. Fujiyama *et al*. unpublished data). The phytophagous ladybird beetle *Henosepilachna vigintioctopunctata* (Fabricius), a voracious feeder on solanaceous crops and weeds, ranges from South and Southeast Asia to Oceania (Richards 1983, Schaefer 1983, Katakura *et al*. 1988, 2001, Jadwiszczak & Węgrzynowics 2003). In several parts of Southeast Asia, it occurs on centro in addition to its usual solanaceous hosts (Nishida *et al*. 1997, Shirai & Katakura 2000). Schultze-Kraft & Clements (1990) noted that another *Solanum*-feeding *Henosepilachna* species, *H. indica* (Mulsant), utilises centro in Malaysia, although this record needs confirmation because it can be very difficult to identify Asian *Henosepilachna* species by their external morphology. In any case, except for *H. vigintioctopunctata* and *H. indica,* there is no record of *Henosepilachna* feeding on centro in the wild, and information is lacking on whether other Asian *Henosepilachna* species can utilise centro. Here we report the cryptic, incipient ability of a population of *Henosepilachna diffinis* (Eydoux be Souleyet) to use centro. This beetle also uses solanaceous plants as major hosts and occasionally co-occurs with *H. vigintioctopunctata*.

MATERIALS AND METHODS

Henosepilachna diffinis is supposedly distributed in the Philippines, Borneo and Java (Jadwiszczak & Węgrzynowics 2003), although we have not found this species in Java in our intensive studies on epilachnine ladybird beetles. Adult beetles used in our study were collected on *Solanum* sp. (Solanaceae) in early August 2007 at Taman Wisata Alam Bukit Bangkirai, East Kalimantan, Indonesia (01º01'42"S, 116º52'01"E). All rearing experiments were carried out at the Research Center for Biology, Cibinong, West Java. The rearing conditions were not controlled but were relatively constant, approximately 30°C (ranging from 27 to 33°C), 54% RH (38 to 71%), and 12L:12D (because of the location near the equator). Host plant leaves used for experiments were collected at Cibinong.

Feeding choice tests

Feeding choice tests using adult beetles were carried out to evaluate feeding preference for centro relative to the solanaceous plant *Solanum torvum* Sw., one of the common hosts of *Henosepilachna* beetles feeding on solanaceous plants in Indonesia (Kalshoven *et al*. 1981, Katakura *et al*. 1988, 2001).

Two pieces of leaves (each about 12 cm^2), one from centro and one from *S. torvum*, were placed in a transparent polystyrene container (5.5 × 6.0 × 2.0 cm), the bottom of which was lined with moist filter paper. A beetle was released into the container and allowed to feed freely on leaves for 24 hours. Each beetle was tested twice, on each of two successive days. Seven females and eight males were examined.

After the test, the leaves were photocopied and scanned into a computer. The areas of leaves consumed by each beetle were measured with the image processing software NIH Image, ver. 1.63 (National Institute of Health, Bethesda, MD, USA) and Photoshop CS4 Extended (Adobe Systems Incorporated, San Jose, CA, USA). Because feeding scars by herbivorous ladybird beetles have an unique lace-like appearance (cf. Howard 1941), the measurement of consumed area was possible without a pre-measurement of area of leaves provided to respective beetles. Area consumed was converted to wet weight (mg) to minimize possible bias due to differences in leaf thickness or water content between the plant species.

The mean amounts consumed during per day were calculated for each beetle and host plant. Differences in preference for the two plant species were analyzed separately for each sex by *t*-tests for paired comparisons.

Rearing of larvae

Female beetles used in the feeding tests described above were checked daily for oviposition, and egg masses were collected. Six egg masses produced by six different females were collected, and four to 20 newly hatched larvae were obtained from each mass (85 larvae in total). The larvae from each egg mass were divided equally into two groups, and each group was reared on leaves of either centro or *S. torvum*; in total, 42 larvae were reared on centro and 43 on *S. torvum*. Larvae were reared individually in transparent polystyrene containers identical to those used in the feeding choice tests. Fresh leaves were provided *ad libitum* throughout the experiment. Individuals were reared until they reached adulthood or died. Larvae were checked daily for survival and molting up to the 4th (final) instar, pupation, and subsequent adult emergence. The feeding response of each larva was also recorded, *i.e.*, whether or not it fed on the leaves offered.

Differences in the feeding response to the two plants offered were analysed by logistic regression analysis. Survival curves during larval development on the two plants were drawn with the Kaplan-Meier method and compared by using the generalised Wilcoxon test. These analyses were conducted using the statistical software JMP ver. 6.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Feeding choice tests

Adult beetles of both sexes significantly preferred *S. torvum* to centro (Fig. 1). All 15 beetles ate *S. torvum*, and eight of them (six females and two males) also fed on centro. All beetles that ate both plants consumed more *S. torvum* than centro (Fig. 1).

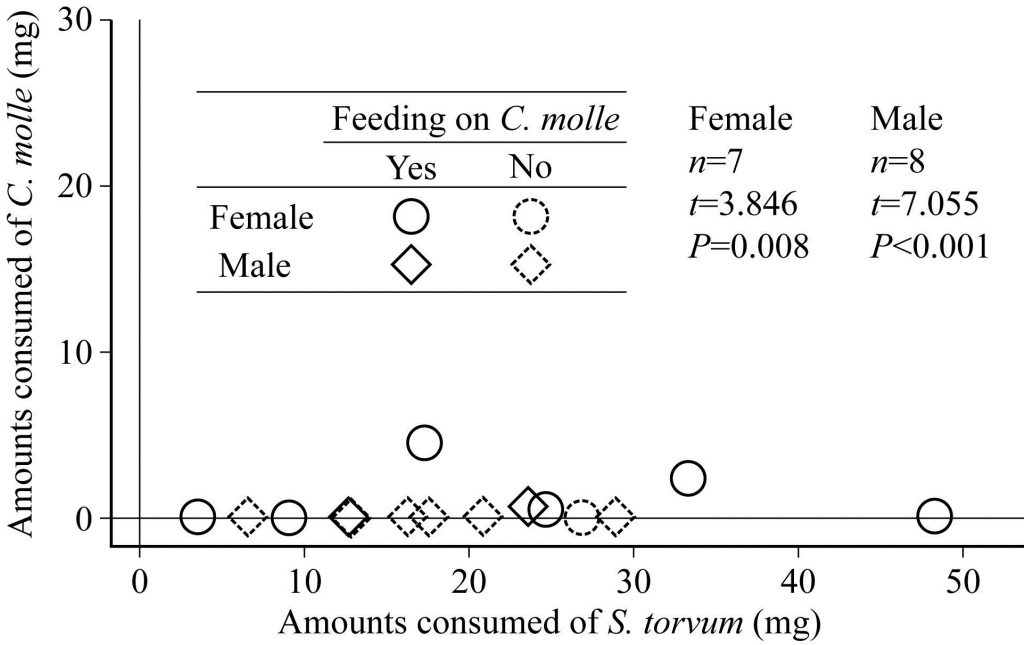

Figure 1. Amounts of *Centrosema molle* and *Solanum torvum* leaves consumed when offered simultaneously to *Henosepilachna diffinis* adults. Symbols indicate whether *C. molle* was consumed, by males and females. The *t*-test for paired comparisons was used to evaluate the preference for either plant, separately for each sex. Both males and females ate significantly more *S. torvum* than centro.

Rearing of larvae

Approximately 80% of the first instar larvae fed on centro (Fig. 2), but only about 20% of them survived to the second instar and none reached the pupal stage on centro (Fig. 3). By contrast, mortality on *S. torvum* was very low throughout all developmental stages; nearly all first-instar larvae ate *S. torvum* and approximately 90% of larvae survived to adulthood (Fig. 3). Significantly fewer first-instar larvae ate centro than *S. torvum* (d.f.=1, χ^2=5.107, P=0.024), and their survival was significantly lower on centro compared with *S. torvum* (Fig. 3; d.f.=1, χ^2=67.791, P<0.001).

DISCUSSION

Both the preference of adult beetles and larval developmental performance indicated that centro is not suitable as a host for *H. diffinis*; adults never preferred centro to *S. torvum*, and no larvae reached the pupal stage on centro. These results are consistent with our field experience that *H. diffinis* has never been collected on centro. However, the results also indicated that *H. diffinis* has a very low but detectable potential to utilise centro; more than half of *H. diffinis* adults ate small

amounts of centro leaves even in the presence of the solanaceous host (Fig. 1); approximately 80% of the hatched larvae showed a positive feeding response on centro (Fig. 2); and some larvae survived to the 2nd or 3rd instar (Fig. 3). It is unknown whether the observed cryptic ability to use centro is restricted to the local population we studied, or a more or less common across *H. diffinis* populations. In any case, because *H. diffinis* does not leave offspring on centro and has not been observed to occur on centro in the wild, the incipient ability of *H. diffinis* to utilise centro is not known in nature. These results suggest that *H. diffinis* already had this ability before the introduction of centro into Southeast Asia.

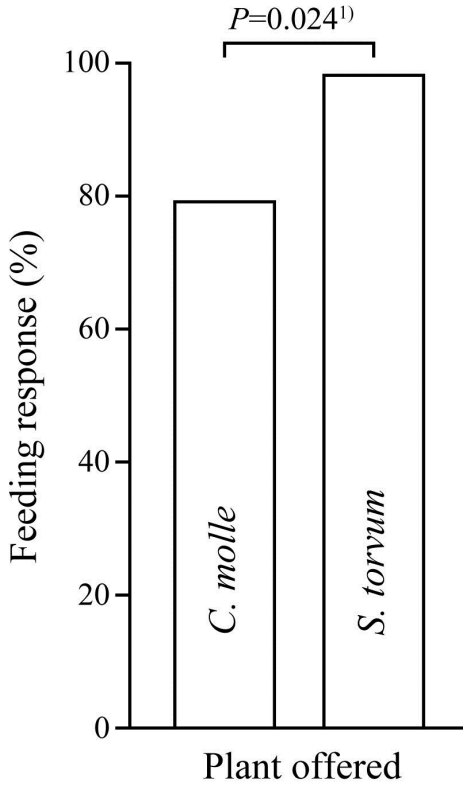

Figure 2. Frequency of *Henosepilachna diffinis* larvae showing a feeding response to *Centrosema molle* and *Solanum torvum*. The difference between the treatments was tested by logistic regression analysis.

As mentioned earlier, another *Solanum*-feeding *Henosepilachna* species, *H. vigintioctopunctata*, now frequently utilises centro in various parts of Southeast Asia (Nishida *et al*. 1997, Shirai & Katakura 2000, Katakura *et al*. 2001, N. Fujiyama *et al*. unpublished). Previous studies indicated that the ability of *H. vigintioctopunctata* to utilise centro has a genetic basis, and populations from localities where centro is more frequently utilised usually have a higher ability to feed and develop on centro (N. Fujiyama *et al*. unpublished). However, how *H. vigintioctopunctata* achieved the ability to utilise centro remains unclear. Our present results suggest that *H. vigintioctopunctata* might have already had a partial ability to use centro, similarly to *H. diffinis*,

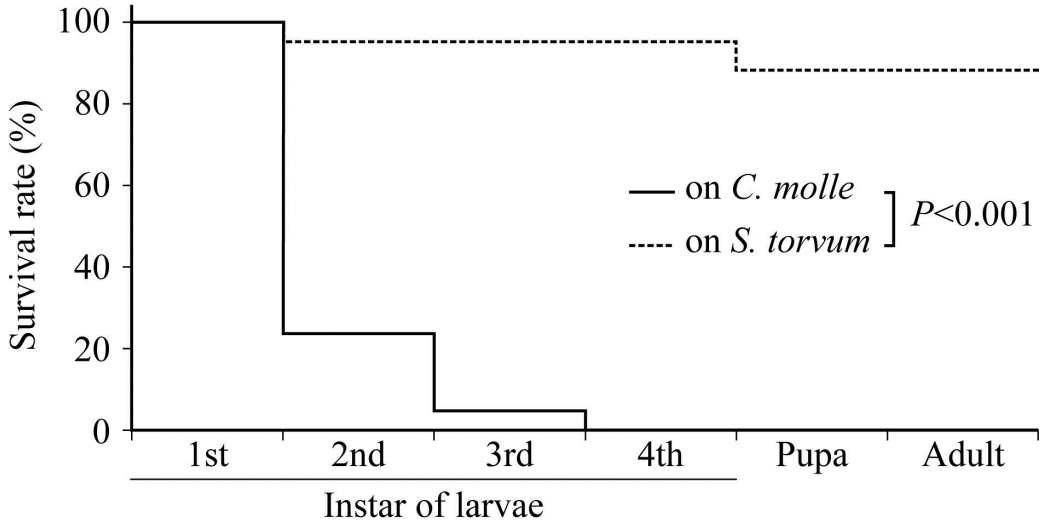

Figure 3. Survival of *Henosepilachna diffinis* on *Centrosema molle* and *Solanum torvum* across developmental stages. The survival curves were drawn with the Kaplan-Meier method and compared by the generalised Wilcoxon test.

when this beetle species encountered centro for the first time, and that this triggered a subsequent host-range expansion of *H. vigintioctopunctata* from solanaceous plants to include centro in various parts of Southeast Asia. Since at least some ability to utilise centro seems shared by *H. vigintioctopunctata* and *H. diffinis* (and *H. indica* as well, according to Schultze-Kraft & Clements 1990), this ability may have originated in the common ancestor of these species. Our results further suggest that other *Henosepilachna* species may also be able to use centro. To understand the adaptation of *H. vigintioctopunctata* to centro, further analyses of the feeding ability of various *Henosepilachna* species would be fruitful, along with molecular a phylogenetic analysis of these species (cf. Kobayashi *et al*. 1998, 2009).

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