Research paper

Oviposition Preference and Larval Survival of *Troides aeacus formasanus* (Lepidoptera: Papilionidae) on *Aristolochia zollingeriana* in Different Environments in the Kenting Area

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【Summary】

Troides aeacus formosanus, an endemic butterfly subspecies of Taiwan, is listed as a protected species of Taiwan. Its population is mainly distributed in the Kenting area of southern Taiwan, where *Aristolochia zollingeriana* serves as the butterfly's main host plant. We surveyed the host plants in the Kenting area, and recorded numbers of eggs laid by female *T. aeacus formosanus*, larval survival rates, and habitat variables. Our goals were to determine the characteristics of sites selected by female butterflies for oviposition, their preferred sites for ovipositing larger egg batch sizes, and habitat variables associated with higher larval survival rates. The results showed that 70% of the 83 identified potential habitat sites were utilized by *T. aeacus formosanus*. Habitats under a closed canopy were more frequently utilized by females than those under an open canopy. Larger host plants growing in the upper level of the forest also had a higher female utilization rate. During the field study, 101 egg batches were identified with a total of 1078 eggs. We found that females preferred to lay larger egg batch sizes on host plants with more leaves, host plants growing in the upper strata of the forest, and host plants by the road edge under an open canopy. Among egg batches, the larval survival rate averaged 5.7% and peaked at 40%. We found that host plants that were growing in the upper level of the forest and with greater leaf abundances hosted egg batches with greater larval survival rates. As for habitat types, although females laid more eggs by the road under an open canopy, their larvae did not have higher survival rates than larvae in other habitat

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Received April 2010, Accepted September 2010. 2010年4月送審 2010年9月通過。

types. Ultimately, vertical stratification and leaf abundance of *A. zollingeriana* were the most important factors influencing female oviposition preference and larval survival rates.

Key words: egg batch size, habitat type, host plant, oviposition site selection, vertical stratification.

Hsieh KJ, Kuo YL, Perng JJ, Lee TC, Lee HL. 2010. Oviposition preference and larval survival of *Troides aeacus formasanus* (Lepidoptera: Papilionidae) on *Aristolochia zollingeriana* in different environments in the Kenting area. Taiwan J For Sci 25(4):353-68.

研究報告

墾丁地區黃裳鳳蝶產卵偏好及幼蟲存活在不同環境的港口馬兜鈴植株的表現

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摘 要

黃裳鳳蝶是台灣的特有亞種，為台灣保育類野生動物之一。台灣南部的墾丁地區是此蝶的主要分布地，而港口馬兜鈴是其在此區最主要的食草。本研究調查墾丁地區生長的港口馬兜鈴食草，監測黃裳鳳蝶母蝶於各食草棲地產下的卵數，以及幼蟲存活率，並調查各棲地的環境條件。研究目的為瞭解此蝶選擇來產卵之處的特性如何，偏好於何處產下較大的卵群，並探討幼蟲存活率較高的棲地條件為何。結果發現，在83處潛在可利用的食草棲地中，有70%被此蝶實際利用，林冠鬱閉棲地被利用的比例顯著高於林冠開放處。生長至森林上層的大型食草被母蝶利用的比例也較高。研究期間共標定此蝶101組卵群，共計1,078個卵。母蝶偏好在生長至森林上層且葉片較多的食草，以及林冠開放的馬路林緣棲地產下較大的卵群。在不同卵群中，幼蟲存活率平均為5.7%，最高可達40%。幼蟲存活率較高的食草，是生長至森林上層，且葉片豐量較高的植株。就棲地類型而言，母蝶產下較大卵群的馬路林緣棲地，其幼蟲存活率並未顯著高於其他類型的棲地。整體而言，食草港口馬兜鈴在森林中的垂直分層及葉片豐量，是影響黃裳鳳蝶產卵偏好及幼蟲存活率最重要的因子。

關鍵詞：卵群大小、棲地類型、食草、產卵棲地選擇、垂直分層。

謝桂禎、郭耀綸、彭仁君、李宗祈、李惠玲。2010。墾丁地區黃裳鳳蝶產卵偏好及幼蟲存活在不同環境的港口馬兜鈴植株的表現。台灣林業科學25(4):353-68。

INTRODUCTION

Troides aeacus formosanus Rothschild 1899 (Papilionidae: Troidine) is a large and beautiful butterfly subspecies endemic to Taiwan. It was frequently observed in low-elevation mountainous areas in the past in the south. However, due to environmental changes and high predation pressure, the population size of *T. aeacus formosanus* drastically decreased from the 1970s, and the butterfly was extirpated from some areas (Yen and Yang 2001). In 1989, *T. aeacus formosanus* was added to the protected species list by the Council of Agriculture and listed in the category of rare and valuable species (Yang 2004). In 2010, the Council of Agriculture re-classified the species into the category of "other conservation-deserving wildlife" (Wu 2010). The current distribution of *T. aeacus formosanus* includes low mountainous areas in southern and southeastern Taiwan, particularly the area around Kenting where high numbers of the butterfly still occur (Yang 2004). In Kenting, the main host plant for *T. aeacus formosanus* larvae is the perennial vine *Aristolochia zollingeriana* (Hsieh et al. 2008). This plant was recently listed as endangered in nature (Lu and Chiou 1997), and its declining distribution and abundance may negatively affect *T. aeacus formosanus* populations. Kenting National Park (NP) staff have attempted to increase *T. aeacus formosanus* natural habitat by nurturing *A. zollingeriana* in nature (Hsieh et al. 2008). However, baseline ecological data concerning *T. aeacus formosanus* are lacking. Information on the niche characteristics of the species is essential for *T. aeacus formosanus* conservation.

Plants and insect species often share an intimate relationship. The selectivity of ovipositing females often provides the initial basis for divergence of insect populations onto different plant species, and it may drive the evolution of some plant defenses (Thompson 1988, Thompson and Pellmyr 1991). Like most troidine butterflies, *T. aeacus formosanus* larvae feed exclusively on plants of the Aristolochiaceae (Rausher 1979, Morais and Brown Jr. 1991, Kondo and Shinkawa 2003). In Kenting NP, species of the *Aristolochia* genus include *A. zollingeriana*, *A. heterophylla*, and *A. cucurbitifolia* (Lu and Chiou 1997, 1998, Hsieh et al. 2008) but *T. aeacus formosanus* larvae feed predominantly on *A. zollingeriana* (Hsieh et al. 2008). *Aristolochia zollingeriana* also hosts *Pachliopta aristolochiae interpositus* and *Byasa polyeuctes termessus* larvae. However, *B. polyeuctes termessus* infrequently feed on *A. zollingeriana*, so *P. aristolochiae interpositus* is the main competitor of *T. aeacus formosanus* (Hsieh et al. 2008).

Oviposition behavior contributes to the evolution of host preference and specificity of insects (Thompson and Pellmyr 1991). Females of different insect species feeding on the same host plant display different oviposition preferences (Konvička and Kuras 1999, Bergström 2005). Vertical stratification (Fermon et al. 2003, 2005) and vegetation structure (Morais and Brown Jr. 1991, Beccaloni 1997, Bergman 2002, Anthes et al. 2003, Bergström 2005) both influence oviposition site selection. In a variable environment, consistency of a particular habitat may also influence oviposition site selection (William and Resetarits 1996).

Previous studies indicated that some butterfly species prefer sunny and open habitats for oviposition (Rausher 1979, Konvička and Kuras 1999, Bergström 2005), while others prefer shady areas (Rausher 1979). Unlike adults, larvae are unable to migrate freely in search of host plants and are consequently constrained to feed at the hatching site (Morais and Brawn 1991, Konvička and Kuras 1999, Bergström 2005, Doak et al. 2006). Therefore, oviposition site selection is a crucial indicator of the suitability of a plant for larval growth and development (William and Resetarits 1996). Larval survival is strongly dependent on habitat characteristics (Fred and Brommer 2003, Anthes et al. 2008). Habitat quality is the main factor that influences the probability of butterfly extinction (Li et al. 2006). For butterflies, habitat quality can consist of larval host plants, suitable microclimates, nectar sources, and particular soil nutrient compositions (Fred and Brommer 2003). Some empirical studies indicated that female oviposition preference is not based solely on the availability of host plants. Rather, females may prefer sites better suited to larval survival (Thompson and Pellmyr 1991, Bergström 2005, Doak et al. 2006, Wiklund and Friberg 2008). Other studies suggested that oviposition site discrimination accrues additional benefits: eggs or larvae may experience reduced predation, parasitism, or lower intra- or interspecific competition at some sites (Rausher 1979, Thompson and Pellmyr 1991, Doak et al. 2006, Wiklund and Friberg 2008). Still, other studies demonstrated that while some larval performances follow the female preference, in other cases they do not (Rausher 1979, Thompson and Pellmyr 1991, Albanese et al. 2008).

In a field study observing habitat utilization by *A. zollingerian* in closed versus open canopy, *T. aeacus formosanus* preferred closed-canopy habitats (Hsieh et al. 2008). The study also observed plant responses to larval feeding and discovered that females moved to different habitats for oviposition according to the changing abundances of host plants.

In this study, we identified the host plant characteristics and habitat variables that influence oviposition site selection, oviposition preference, and larval survival of *T. aeacus formosanus*. We addressed the following research questions.

1. What host plant and habitat characteristics are selected by *T. aeacus formosanus* for oviposition?
2. What host plant and habitat characteristics are associated with different egg batch sizes and larval survival rates?
3. Do habitats with the largest egg batch sizes also have significantly higher larval survival?

We hypothesized that habitats with the largest batch sizes will have correspondingly higher larval survival rates.

Addressing these research questions will enable us to identify combinations of host plant and habitat characteristics most suitable for *T. aeacus formosanus* in nature. Furthermore, we will be able to identify environmental factors that are essential for *T. aeacus formosanus* larval survival. The study findings will contribute to knowledge of *T. aeacus formosanus* ecology and form the foundation for *T. aeacus formosanus* conservation.

MATERIALS AND METHODS

Study area

The study region was situated within the Kenting NP (21°57’N, 120°49’E). We conducted an intensive foot survey throughout the approximately 3600-ha forest area to locate *A. zollingeriana* vines in the field. The plants are patchily distributed in Sheding, Siangjiao Bay, Shadao, Erluanbi, and the easten coast of the Erluanbi Peninsula, and cover approximately 206 ha of forest (Fig. 1). According to the Central Weather Bureau of Taiwan, in 1999~2008, the average annual rainfall in the Kenting area was 2114 mm. From October to April, a dry season occurs with a strong northeasterly monsoon, while between May and September, there is a wet season characterized by high rainfall and typhoons. There are obvious distinctions between the dry and wet seasons. The lowest temperature was recorded in January (19.1℃), and the highest in July (27.2℃). The average annual temperature was 23.6℃ (Lu et al. 2004).

Elevations within the study area range 10~300 m, and the major vegetation type is uplifted coral reef forest. Vegetation structures in the upper levels of the forest are influenced by the winter monsoon. In comparison to shrubs on top of uplifted reef rocks, plants growing in crevices between reef areas appeared tall and straight, thus demonstrating that vegetation structure is associated with micro-geographic factors (Su and Su 1998,

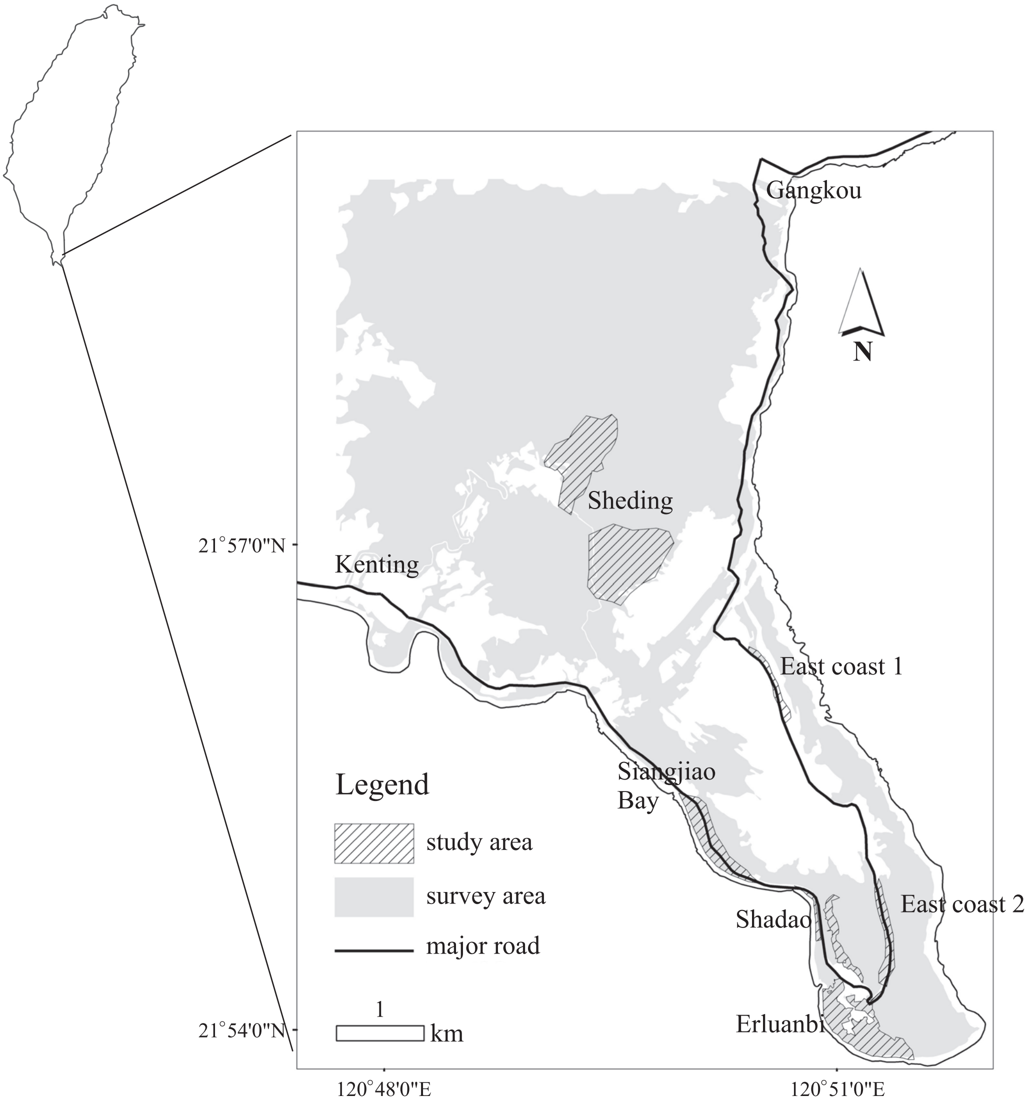

Fig. 1. Location of the study areas (206 ha) in the Kenting region.

Wang et al. 2004). Areas with frequent human presence are dominated by secondary vegetation and plantations.

Host plant characteristics and habitat envionmental conditions

We located every individual *A. zollingeriana* vine in Kenting region habitats by walking along search transects. In total, 122 plants were identified. A few (*n* = 39) of the identified vines were dead either due to natural or human causes, or were growing in the upper reaches of the uplifted reef rocks beyond our monitoring capabilities. Eighty-three (83) *A. zollingeriana* vines were suitable for ongoing observations.

We recorded the plant size, ground level vine diameter, leaf abundance, and vertical stratification of each host plant. We also recorded 6 environmental variables for each plant site: habitat type, soil substrate, elevation, tree height, larval competitor presence, and monsoon damage. The detailed specification of these host plant characteristics and habitat variables are listed in Table 1. From here onwards, "host plant" refers to *A. zollingeriana*, also as the "site". Habitats for *T. aeacus formosanus* oviposition or larvae include both the host plant and the environment surrounding the host plant.

Hsieh et al. (2008) divided *A. zollingeriana* habitat into open and closed canopies. This study investigated whether accessibility to a closed canopy and the degree of exposure to an open canopy influences oviposition. The habitats were divided into 4 categories based on their condition: road edge, trail edge, inner forest, and forest trail (Table 1).

Oviposition site selection

Between September 2005 and September 2006, field observations were made once every 6~12 d, and between October 2006 and June 2007, observations were made on a monthly basis. At each observation, we recorded the leaf abundance and vertical stratifi-

Table 1. Characteristics of the host plant, *Aristolochia zollingeriana*, and environmental variables at *Troides aeacus formosanus* larval habitats

Parameter	Classification	Specification
Host plant characteristics		
plant size	large, small	Vine length > 2 m was classified as large, < 2 m was classified as small.
ground diameter		Diameter measured 5 cm above ground.
vertical stratification	canopy, midstory, understory	Canopy was defined as within 1 m of the tree top, understory was defined as within 1 m of the ground, with the remainder defined as midstory.
leaf abundance		Depending on the research question, leaf abundance was either the average leaf abundance during the study period or leaf abundance during oviposition.
Environmental factors		
habitat type	road edge, trail edge, inner forest, forest trail	Road edge: habitats within 5 m of a road. Trail edge: habitats within 5 m of a trail. Inner forest: habitats in a completely closed canopy. Forest trail: habitats under continuous canopy cover but within 5 m of a trail. The former 2 categories are classified as open canopy and the latter 2 are closed canopy. A road had a width of > 6 m, while a trail was < 4 m.
major substrate	coral reef, soil, shallow soil around boulder	
winter monsoon	wind exposed, wind protected	Wind-exposed forest displayed the wind-cut phenomenon. This was absent in wind-protected forest.
competitor	yes or no	Competitors included *Pachliopta aristolochiae interpositus* and *Byasa polyeuctes termessus*.
elevation		Range of elevation in study area: 10~300 m.
tree height		Height (m) of trees associated with *A. zollingeriana*.

cation of each of the 83 potential *A. zollingeriana* host plants. In addition, we determined whether the host plant or its surrounding environment had eggs, larvae, or pupae of *T. aeacus formosanus* or its competitors. We climbed the trees or set up ladders (2.5~6 m) to make observations in the upper canopy.

We defined *A. zollingeriana* as an oviposition site "selected" by *T. aeacus formosanus* by the presence of *T. aeacus formosanus* eggs or larvae at the plant. On the other hand, plants with no eggs or larvae were considered "not-selected" sites. We also defined a site as "utilized" if it was selected by a female for oviposition. Analysis was undertaken to determine similarities and differences in various factors among sites that were either selected or not selected as an oviposition site. Leaf abundance was calculated as the average of all observations.

Oviposition preference and larval survival

Between July 2004 and September 2006, observations were made every 6~12 d, and between October 2006 and June 2007, observations were made once a month. At every observation, we labeled each new egg and recorded the number of new eggs laid by *T. aeacus formosanus* on each plant. We continued to count the number of eggs until there were no new eggs and considered all eggs up to that point to be 1 batch. Habitats with the largest batches were considered to be the preferred oviposition sites for females; while sites with high egg-to-emergence ratios were considered to be favorable for larval survival.

During the study period, 101 egg batches were recorded. Hatching, larval growth, pupal counts, and successful emergences were recorded in order to determine egg-to-emergence survival rates. Leaf abundance was taken as the number of leaves present at the time of oviposition.

Analysis methods

Habitat variables included both discrete variables, such as plant size, vertical stratification, habitat type, soil substrate, and larval competitor, and continuous variables, such as vine diameter, leaf abundance, elevation, and tree height. We therefore applied a general linear model (GLM) to analyze the effects of habitat variables on *T. aeacus formosanus* oviposition site selection, oviposition preference, and larval survival. Some of the continuous variables were highly correlated, analysis of covariance (ANCOVA) was applied to adjust the correlated variables, and significant differences within discrete variables were analyzed by the GLM. Pearson correlation coefficients were calculated to determine correlations among variables. When there was a significant difference within the discrete independent variables of the GLM, Fisher's least significant difference (LSD) test was used to calculate the confidence interval of the pairwise comparisons. A Chi-squared analysis was used solely to determine whether significant differences in the total egg batch numbers and egg numbers existed among the 4 different habitat types. Statistical significance was set to $p < 0.05$.

RESULTS

Oviposition site selection by *T. aeacus formosanus*

Of the 83 potential host plants for *T. aeacus formosanus*, only 58 (or 70%) were selected for oviposition. Comparisons across the 10 measured variables were made to determine whether significant differences existed between plants selected and not selected by females for oviposition. The results indicated that significant differences existed between plants in terms of plant size, vine diameter, vertical stratification, and type of habitat (Table 2). The respective percentages of available large and small plants were 80 and 20%. However, of the plants selected by females, 97% were large and 3% were small. Host plant diameter was significantly positively correlated with female oviposition site selection ($r = 0.321$, $p < 0.01$). In terms of vertical stratification, host plants growing in the canopy, midstory, and understory levels accounted for 57, 25, and 18%, respectively. However, of the 58 plants selected as oviposition sites, none was from the understory. Percentages of host plants selected that were located in the canopy and midstory levels were 74 and 26%, respectively. Oviposition site selection between different strata significantly differed (Fig. 2). In terms of habitat type, numbers of host plants found in each of the 4 habitats (road edge, trail edge, inner forest and forest trail) were 23, 20, 13, and 27, respectively. However, of the 58 plants utilized as oviposition sites, 12, 10, 12, and 24 were in these respective habitat types. Habitat types of inner forest and forest trail both had utilization percentages significantly higher than those of road-edge and trail-edge habitat types (Fig. 3). As a result, habitats under a closed canopy were more frequently utilized by females than those under an open canopy. Large host plants, which grow up to

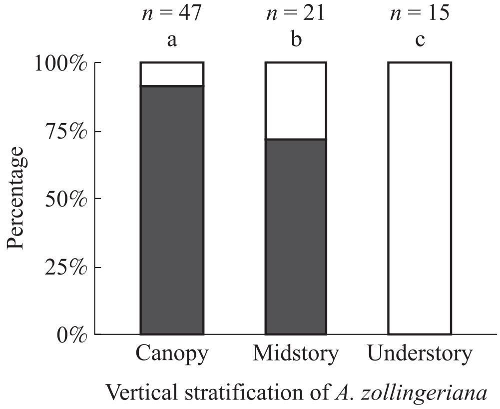

Fig. 2. Proportion of *Aristolochia zollingeriana* plants utilized (■) and not utilized (☐) by female *Troides aeacus formosanus* in each vertical stratification level. Significant differences in utilization by vertical stratification are indicated by labels (a, b, and c) (Fisher's LSD, $p < 0.05$). For definitions of vertical stratification please refer to Table 1.

Table 2. Statistical analysis (GLM) showing correlations of female *Troides aeacus formosanus* oviposition site selection, batch size (egg numbers per batch), and larval survival with *Aristolochia zollingeriana* characteristics and environmental variables

Parameter	Oviposition site selection		Egg batch size		Larval survival	
	F value	*p*	*F* value	*p*	*F* value	*p*
Plant size	6.66	**	3.38	n.s.	0.93	n.s.
Ground diameter	4.78	*	0.66	n.s.	0.02	n.s.
Vertical stratification	56.16	***	9.14	**	4.19	*
Leaf abundance	1.71	n.s.	115.68	***	65.30	***
Habitat type	10.24	***	5.89	**	1.58	n.s.
Substrate	0.10	n.s.	0.70	n.s.	0.56	n.s.
Winter monsoon	0.19	n.s.	0.00	n.s.	0.06	n.s.
Competitor	0.38	n.s.	3.43	n.s.	0.76	n.s.
Elevation	0.69	n.s.	2.69	n.s.	0.97	n.s.
Tree height	1.83	n.s.	1.32	n.s.	0.20	n.s.

n.s., not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

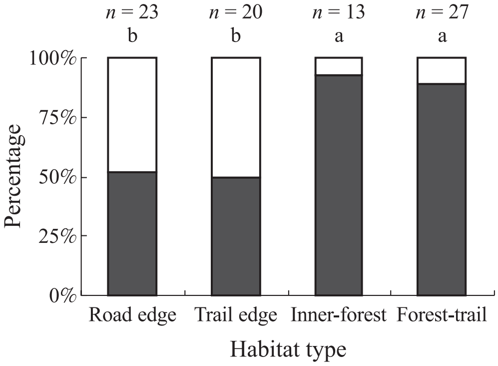

Fig. 3. Proportions of utilized (■) and unutilized (□) habitats by female *Troides aeacus formosanus* across 4 habitat types. Labels "a" and "b" indicate statistically significant differences between groups (Fisher's LSD, $p < 0.05$). For definitions of habitat types refer to Table 1.

the canopy level of the forest, were also more frequently selected by females.

Oviposition preference

During the observation period, 101 egg batches of *T. aeacus formosanus* were identified with a total egg number of 1078. A wide range in egg numbers per batch (1~68) was observed, with an average of 10.7±1.2 (mean ±SE). Data on eggs per batch were divided into categories, with 1~10 eggs/batch being the most common (67%, Fig. 4). Forty-six percent of the total batches had fewer than 5 eggs, and only 1 batch had more than 50 eggs.

Vertical stratification, habitat type, and leaf abundance were significantly correlated with egg batch size (Table 2). In terms of vertical stratification of the host plants in the forest, plants in the understory were not utilized for oviposition, while 76 and 24% of the 101 egg batches were distributed in the canopy and midstory levels, respectively. In terms of the number of eggs laid, 91% of eggs were located on host plants in the canopy level.

Egg batches were distributed across the 4 habitat types (road edge, trail edge, inner forest, and forest trail) in the respective proportions of 29, 20, 26 and 25%, revealing a significant difference among habitat types ($x^2 = 9.34$, $df = 3$, $p < 0.05$). Due to the different numbers of host plants, the average batch numbers per host plant in each of the 4 respective habitat types were 1.3, 1.0, 2.0, and 0.9 batches: the highest was located in the inner forest habitat (Fig. 5a). In terms of the number of eggs laid, the distribution across the 4 habitat types described above was 43, 21, 19, and 17% respectively (Fig.

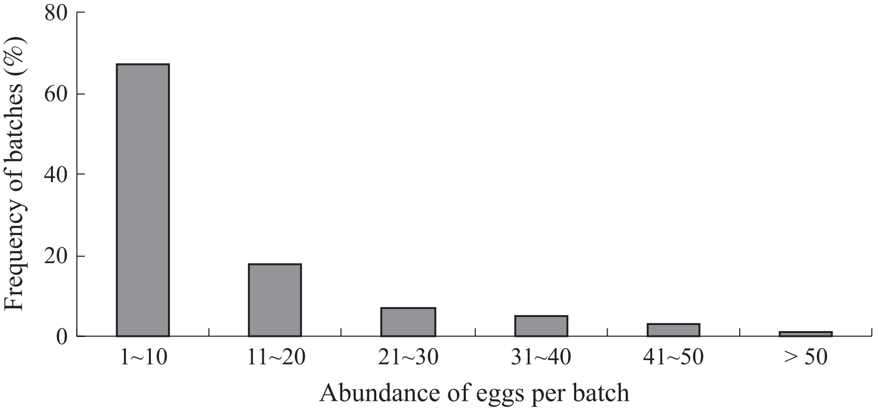

Fig. 4. Frequency distribution of batch sizes of *Troides aeacus formosanus* for 101 batches.

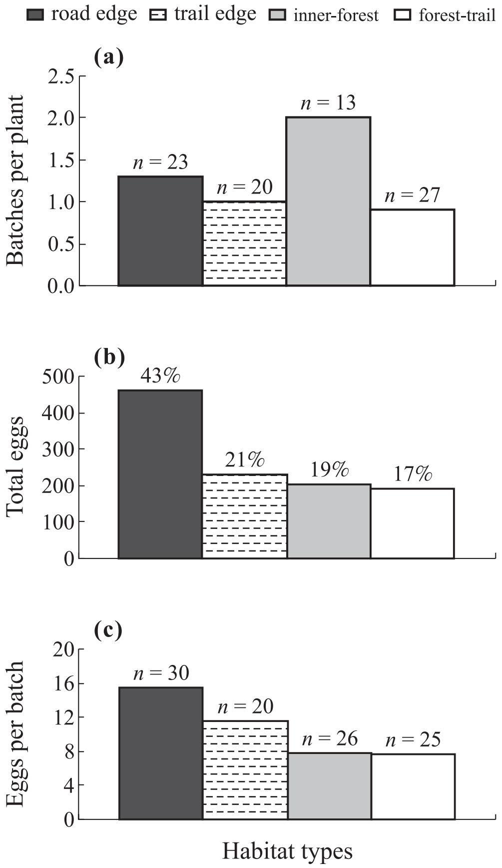

Fig. 5. Average number of *Troides aeacus formosanus* batches per host plant (a), total number of eggs (b), and average number of eggs per batch (c) across the 4 habitat types.

5b), revealing a significant difference across the 4 habitats ($x^2 = 170.49$, $df = 3$, $p < 0.001$). The average numbers of eggs per batch across the 4 habitat types were 15.3, 11.6, 7.7, and 7.5, respectively (Fig. 5c). Road-edge habitat had a significantly higher average number of eggs per batch (larger batch size) compared to other habitats, indicating a preference among females for road-edge habitat. In addition, leaf abundance showed a significant positive correlation with egg numbers ($r = 0.777$, $p < 0.0001$).

Larval survival and habitat environmental conditions

Of the observed 101 egg batches, 65 batches of larvae failed to emerge, with 0% survival. Only 36 batches of larvae successfully emerged to butterflies. In all egg batches, the highest egg-to-emergence survival rate was 40%, with an average of 5.7±0.9% (mean±SE).

Of the 10 characteristics of the host plant and habitat environment, only vertical stratification and leaf abundance showed a significant relationship with larval survival (Table 2). Ninety-six percent of successful emergences were located in the canopy, while 4% were in the midstory. In addition, leaf abundance showed a significant positive correlation with larval survival ($r = 0.677$, $p < 0.0001$). The analysis also showed no significant difference in larval survival rates among the different habitat types ($F = 1.58$, $df = 3$, $p = 0.199$) (Table 2).

DISCUSSION

Oviposition site selection by *T. aeacus formosanus*

During field observations, 70% of *A. zollingeriana* plants were utilized by *T. aeacus formosanus*, while 30% remained unutilized. This demonstrates that even in locations where the host plant was scarce, females were selective regarding oviposition sites. Ninety-seven percent of females chose larger plants for oviposition. This oviposition site selection reflects females' tendency to fly above the canopy when searching for an oviposition site (Hsieh et al. 2008) and the camouflage provided by the vegetation structure (Morais and Brown 1991, Beccaloni 1997, Bergman 2002, Anthes et al. 2003, Bergström 2005). In the present study, we found that the majority (68%) of large host plants grew in the canopy level of the forest, providing relatively accessible oviposition sites for females. Anthes et al. (2003) found that the probability of a host plant being accepted by a female marsh fritillary butterfly (*Euphydryas aurinia)* for oviposition increased with plant size, but decreased with vegetation density. In the present study, we found that host plants in the understory of the forest were not utilized by female *T. aeacus formosanus* for oviposition (Fig. 2) due to the vegetation density and the height of the tree on which *A. zollingeriana* was found. In the Kenting region, the average tree height with which *A. zollingeriana* was associated was 4.1 ± 1.8 m (Hsieh et al. 2008). Hence, the understory level in our study was approximately 3 m below the normal flying height of *T. aeacus formosanus*. Females were unlikely to descend to the understory level for oviposition due to obstruction by branches and leaves.

Of the 4 habitat types investigated in this study, female *T. aeacus formosanus* preferred inner-forest and forest-trail habitats. This was shown by the significantly higher use of host plants within these closed-canopy habitats for oviposition (Fig. 3). This finding is consistent with a proposal by Hsieh et al. (2008), which posited that "closed canopy type habitats had a higher utilization frequency by *T. aeacus formosanus* compared to open-canopy type habitats." In this study, the inner-forest habitat had the highest utilization frequency by *T. aeacus formosanus* (92%, Fig. 3). However, host plants found within this habitat represented only 16% of the total host plants identified in the study. In the Kenting region, due to road construction, agricultural needs, and recreational needs, only a small portion of the original undisturbed natural forest remains. The rest of the region contains plantations or secondary forests (Chen 1985), which fragment the original forest structure. This fragmentation has changed the distribution of *T. aeacus formosanus*. Of the 83 host plants of *A. zollingeriana* identified in this study, 8 were identified with the aid of local residents. These eight plants were large in size and at least 30~40 yr old. When they were first discovered by local residents, the plants were all in closed-canopy forest. However, due to agricultural and developmental needs, the surrounding forests were destroyed, leaving the plants on the forest edge. Many *A. zollingeriana* have disappeared due to human activities. Thus one can postulate that the low number of *A. zollingeriana* found in the inner-forest habitat is due to destruction of the original closed-canopy forest.

Oviposition preference

Prior studies found that egg batch size is dependent on host plant characteristics. Bergström (2006) found that female *Polygonia c-album* preferentially deposited larger batches on host plants that had more-favorable characteristics. Thus batch size served as an indicator of host plant quality. In the present study, we found that 46% of the 101 *T. aeacus formosanus* egg batches had fewer than 5 eggs per batch, indicating that the general host plant quality in Kenting region may be poor. Another study indicated that during conditions of surplus host plants, females deposit a majority of eggs at the preferred oviposition site (Thompson and Pellmyr 1991). A number of butterfly species prefer to lay their eggs in open, sunny habitats (Rausher 1979, Grossmueller and Lederhouse 1985, Konvicka and Kuras 1999, Bergström 2005). In this study, we found that female *T. aeacus formosanus* preferred road-edge habitats as demonstrated by the number of eggs laid (Fig. 5b) and egg batch size (Fig. 5c). These habitats are generally open, sunny habitats. Female butterfly activity is largely constrained by environmental factors (Doak et al. 2006). The vegetation structure near road edges in our study was open, and leaf abundance was comparatively higher. In addition, road-edge habitat was easily accessible to females for oviposition. Therefore, habitats exhibiting these characteristics were where the largest numbers of eggs were laid. However, while the females laid more eggs at road-edge habitats (Fig. 5b), the average number of egg batches per host plant was greatest in inner-forest habitats (Fig. 5a). This phenomenon may be attributed to the higher numbers of eggs in road-edge habitats leading to overgrazing of the host plant by larvae, causing long-term plant damage and a longer lag period for the leaves of host plant to regenerate. Therefore, a longer time may occur between 1 batch and the female's return to lay the next batch on the host plant, resulting in fewer batches per plant. In contrast, host plants in the inner-forest habitats maintain consistently high leaf abundances, and may thus be more-frequent oviposition sites for female *T. aeacus formosanus*.

Leaf abundance is a key factor influencing the quantity of eggs laid per batch (Rausher 1980). Doak et al. (2006) discovered that female *Pieris virginiensis* flew over a large number of host plants without ovipositing, and chose to oviposit in only 50% of the host plants they inspected. Field observations from the present study indicated that females did not always immediately engage in oviposition upon the discovery of a suitable host plant, and they laid only a small number of eggs at 1 time. Sometimes the females departed only to return later to lay more eggs. We postulated that the females first responded to micro-environmental factors, and then, based on leaf abundance and potential space for oviposition, laid a certain number of eggs. However, if no other suitable oviposition sites were found, a female returned to lay more eggs. Therefore, habitats with higher numbers of eggs had batches that were often not laid in a single event, but rather over multiple visits. When the *T. aeacus formosanus* population size was relatively large, eggs in the same batch might not have been laid by the same female. When host plants were limited, multiple females were simultaneously in search of suitable host plants and chose to lay the greatest number of eggs in habitats with high leaf abundances. Thus, although leaf abundance did not show a direct relationship with oviposition site selection, it had a significant positive correlation with egg batch size (Table 2). Doak et al. (2006) also indicated that reproductive success was often constrained by oviposition opportunities for female butterflies. In our field observation, we noted that in the spring of 2006, there was a population increase in *T. aeacus formosanus*. By mid-June, there was a scarcity of *A. zollingeriana* leaves due to larval feeding. Thus, although there were active females present during mid-June, no eggs were laid due to a lack of suitable host plants. By the end of June, there were only 3 batches (with a total of 4 new eggs) recorded in our study area (Hsieh et al. unpubl. data).

Female *T. aeacus formosanus* had a behavioral tendency to fly at the canopy level of the forest and engage in oviposition. Due to obstruction by branches, females could only oviposit on leaves that were growing at the canopy level. Therefore, the portion of eggs laid on host plants at the canopy level was 91% of all eggs laid, and host plant vertical stratification in the forest was another key factor influencing female oviposition preference.

Larval survival and suitable microhabitat conditions

Several studies proposed that female oviposition preference is dependent on choosing habitats that are most suitable for larval growth and survival (Rausher1979, Doak et al. 2006, Wiklund and Friberg 2008). In some studies, the definition of a "suitable" host plant for larvae was based on the female oviposition preference (Wahlberg et al. 2002, Anthes et al. 2003, 2008). Doak et al. (2006) showed that female *Pieris virginiensis* oviposition preference corresponded with high larval survival. However, the present study found that in habitats with high numbers of eggs and larger batch sizes (road-edge habitat), larval survival was not significantly higher than in the other 3 habitat types studied. Rausher (1979) reported that all 3 species of female Troidine showed a preference for sunny, open habitats, despite the fact that larvae of 2 species showed higher survival rates in shaded habitats. This suggests that female oviposition preference is not always consistent with maximum larval survival. In the present study, we also found that female *T. aeacus formosanus* oviposition preferences did not necessarily confer survival advantages on the larva. Thus, our results did not support the hypothesis proposed in the literature.

Thompson and Pellmyr (1991) indicated that a lack of correlation between oviposition preference and larval performance may be due to the preferred host plant being rare, or favorable for larval growth under some conditions, but growing in a habitat unfavorable for flight of ovipositing females. The host, *A. zollingeriana*, in Kenting is already rare (Lu and Chiou 1997). In the present study, there were even fewer *A. zollingeriana* (only 13) growing in the inner-forest habitat, which females preferred for oviposition. Furthermore, we found that *T. aeacus formosanus* females laid more eggs in open habitats such as road edges, which are certainly more favorable for flight.

Habitat destruction and resulting habitat fragmentation due to human activities have major consequences on insect oviposition site selection. Without considering the impacts of habitat fragmentation on oviposition site selection, it would be difficult to assess the effect of fragmentation on offspring fitness (Gibbs and Dyck 2009). Our field observations of *T. aeacus formosanus* oviposition behavior, interviews with local residents, and data analysis, suggest that the destruction of the original Kenting forest and resulting fragmentation have led to *T. aeacus formosanus* females laying more eggs in more-open forest types. However, larval survival in these habitats was equivalent to other habitats. Female oviposition preference did not confer a survival advantage on offspring. Albanese et al. (2008) studied an endangered butterfly species, *Callophrys irus*, and found that canopy coverage was an important microenvironmental factor in the survival of larvae. Late instar larvae survival was dependent on a favorable microenvironment and not on female oviposition preference. Without canopy cover, even in areas of high host plant density, larval numbers were low (Albanese et al. 2008). We found that females laid more eggs in habitats along forest edges, according to leaf abundances. However, canopy cover was reduced in these habitats. In addition to human disturbance that often damages the host plant in open canopy habitats (Hsieh et al. 2008), the microenvironment was not conducive to larval survival.

Several studies showed that selective female oviposition confers the advantages of decreased predation, parasites (Thompson 1988, Thompson and Pellmyr 1991, Doak et al. 2006), and intra- or interspecific competition (Doak et al. 2006). In forests with intact canopies, a female's preference for open habitats may bring advantages of reduced larval predation (Wiklund and Friberg 2008). However, laying greater numbers of eggs along road edges may lead to increased intraspecific competition. Moreover, the butterfly *P. aristolochiae interpositus* in our study area has a high utilization of open-canopy habitats (Hsieh et al. 2008), which would also increase interspecific competition for *T. aeacus formosanus* in this habitat.

Our study indicated the leaf abundance showed a significant positive correlation with larval survival ($r = 0.677$, $p < 0.0001$). The amount of host plants available in a specific habitat was previously shown to be an important factor in larval fitness (Fred and Brommer 2003). Females prefer sites with more leaves, as such sites ensure sufficient food resources for larvae until they reach the pupal stage (Anthes et al. 2003). In times of host plant shortage, females choose to lay fewer eggs on host plants with lower leaf abundances. In our study, over half of the batches that had fewer than 10 eggs per batch, and a 0% survival rate was found on host plants with fewer than 50 leaves. The larvae did not have sufficient food resources and did not survive to emergence.

CONCLUSIONS

To answer the 3 questions we raised in our study, we found that when considering only oviposition site selection, females preferred larger host plants, which grew in the canopy of the forest, and closed-canopy habitats for oviposition. If we only consider the size of the egg batches laid, females preferred open sites with high leaf abundances and easy accessibility, such as habitats along road edges. This phenomenon may be due to the limited distribution of host plants in closed-canopy areas. In present-day Kenting NP, *A. zollingeriana* plants growing in inner-forest habitats are severely impacted by human-induced deforestation and habitat fragmentation, which may limit the population size of *T. aeacus formosanus*. Furthermore, a significant positive correlation existed between egg batch size and *A. zollingeriana* leaf abundance. This positive correlation was more pronounced for host plants growing in the canopy level and in road-edge habitat.

In our study, vertical stratification of host plants and leaf abundance were 2 factors significantly associated with larval survival. However, despite the increased batch size, road-edge habitat did not show a significantly higher larval survival rate. Hence, female oviposition preference did not confer a survival advantage on larvae. Although survival rates among habitats did not show significant differences, in road-edge habitats, larvae experienced intensified inter- and intraspecific competition and a greater degree of human disturbance. As a result, female oviposition preference did not maximize offspring survival. Thus, we suggest that conservation efforts for *T. aeacus formosanus* in Kenting NP, including rehabilitation of *A. zollingeriana*, be conducted in closed-canopy forests. We also recommend planting larger *A. zollingeriana* plants capable of reaching the canopy level. Together these approaches should reduce intra- and interspecific competition and maintain higher leaf abundances. Only then will conditions be conducive to larval survival.

ACKNOWLEDGEMENTS

Special thanks go to Mr. Ta-Ching Chou of National Taiwan Univ. for his valuable assistance in field surveys, the making of Fig. 1, and critical comments on this article. We also thank Mr. Ming-Shong Pan, A-Guo Pan, Cing-Lian Pan, Sin-Jhong Gong, and Fu-Shong Yang of the Sheding tribe for their assistance in *A. zollingeriana* surveys in the Sheding area.

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