EFFECT OF DIFFERENT HOST PLANTS ON THE LIFE CYCLE OF *PAPILIO POLYTES* CRAMER (LEPIDOPTERA: PAPILIONIDAE) (COMMON MORMON BUTTERFLY)

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Abstrak: Kajian terhadap kitar hidup *Papilio polytes* Cramer (Lepidoptera: Papilionidae) (rama-rama) ke atas empat jenis tanaman perumah, *Citrus aurantifolia* Swingle*, C. hystrix* L*., C. reticulata* Blanco and *Murraya koenigii* Sprengel (daun kari) menunjukkan bahawa peringkat larva yang paling lama berlaku pada perumah *Murraya koenigii.* Jangka masa ini berbeza secara signifikan daripada tiga jenis tanaman limau lain yang diuji. Sungguhpun begitu, jangka masa peringkat pupa pada *M. koenigii* adalah yang paling pendek. Instar yang lebih tua mempunyai *stadium* yang lebih lama berbanding instar yang lebih muda dan kecenderungan ini hampir serupa untuk semua jenis perumah. Tiada perbezaan yang signifikan dikesan pada jangka masa pradewasa (larva dan pupa), saiz (panjang dan lebar) pupa dan ciri-ciri dewasa (panjang badan, panjang antena dan lebar sayap) *P. polytes* terhadap semua jenis tanaman limau (P = 0.05). Walau bagaimanapun, terdapat perbezaan pada larva yang dipelihara dengan *M. koenigii.* Kajian ini menunjukkan bahawa *C. reticulata* dan *C. hystrix* ialah perumah yang lebih sesuai antara empat tanaman yang diuji. Jangka masa larva yang paling singkat direkodkan pada *C. reticulata* dan saiz pupa dan dewasa yang paling besar direkodkan pada *C. hystrix. Murraya koenigii* merupakan perumah yang kurang digemari oleh *P. polytes.*

Abstract: Investigation on the life cycle of *Papilio polytes* Cramer (Lepidoptera: Papilionidae) (common mormon butterfly) reared on four host plant species, *Citrus aurantifolia* Swingle*, C. hystrix* L*., C. reticulata* Blanco and *Murraya koenigii* Sprengle (curry leaf) showed that the butterfly spent the longest larval period on *M. koenigii* which was significantly different from the time spent on all citrus species. The duration of the pupal stage, however, was the shortest on this host plant. Older instars have longer stadia than the younger instars and the trend of increasing stadium was almost similar in all host plants. No significant differences were detected in the duration of immature stages (larva and pupa), the length and width of pupa and the adult characteristics (length of body, antenna and wingspan) among all citrus species (P = 0.05). However, they were significantly different from individuals reared on *M. koenigii.* This study revealed that *C. reticulata* and *C. hystrix* were the most suitable among the four host plants for this butterfly. The shortest larval duration and life cycle were recorded in the *C. reticulata* and the biggest pupae and adults occurred on *C. hystrix. Murraya koenigii* was the least preferred host.

Keywords: *Papilio polytes,* Host Plant, Life Cycle

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INTRODUCTION

The relative success of caterpillars (Lepidoptera) living on different host plant species is determined by a variety of factors including the food quality, phenology and morphology of the host plant, and associated predator loads. These factors vary among habitats, and such variation might account for the restriction of some caterpillar species to particular habitats, when their host plant species are more widespread (Loeffler 1993).

The growth of the larvae, longevity and fecundity of the adult of phytophagous insects are influenced by the nutritional value of the host plant (Bernays & Chapman 1994). Larval development and survival depend on a number of factors. The quality of food determined by nutrient availability and presence of secondary compounds, affects the time required for completion of larval development as well as the size or mass achieved at the end of larval stages. A quantitative analysis of the consumption and utilization of host plants by insect herbivores is a commonly used tool in studies of plant-insect interaction (Scriber & Slansky 1981). Both the time to pupation and pupal mass, are good measures of the suitability of a particular host species for the herbivores (Penz & Araujo 1990).

The *Papilio polytes* (Lepidoptera: Papilionidae) (common mormon butterfly) is a tropical or subtropical papilionid butterfly distributed from Southeast Asia to the Southwestern Island of Japan (Corbet & Pendleburry 1992; Nakayama *et al.* 2003; Nakayama & Honda 2004). Adult butterflies usually feed on flower nectars and mate in open lands, forest-edges and corridor of the forests (Nakayama & Honda 2004).

Eighty percent of the larvae from the genus *Papilio* feed on rutaceous plant. In Japan, Rutaceae feeder *Papilio polytes* specializes on *Toddalia asiatica.* Nevertheless, they also feed on *Citrus depressa, Murraya paniculata, Melicope trypilla, Fagara ailanthoides* and *Euodia meliaefolia.* Other papilionid butterflies are known to live on various host plants including Aristolochiaceae, Rutaceae, Annonaceae, Magnoliaceae, Lauraceae and Apiaceae (Miller 1978a, b citing Nakayama & Honda 2004).

Previously, *P. polytes* were very common in the forests. Their larvae are only known to feed on various species of Rutaceae such as *Murraya, Triphasia, Glycosmis, Aegle, Zanthoxylum, Toddadia, Euodia, Atalantia* and *Poncirus* (Corbet & Pendlebury 1992). Interestingly, this butterfly has become urbanised recently. It is common in neighbourhoods with gardens and villages where citrus are grown and no longer easy to find in forest lands (Corbet & Pendlebury 1992; Otsuka 2001). In view of this adaptation, the larva of *P. polytes* has the potential to be a serious pest in citrus orchards.

A study on the effect of food on the biology of an insect is of particular importance in understanding host suitability of plant infesting species and evaluating the magnitude of injury to the crop attacked. With this knowledge, more economical control strategies may be designed accordingly. Various studies have evaluated host plant effect on growth and development of butterflies (Astuti 1993; Nylin & Janz 1996; Janz & Nylin 1997; Barros & Zucoloto 1999; Catta-Preta & Zucoloto 2003). This study was focused on the

growth performance of immature stages of the common mormon butterfly on three species of commercially grown citrus and a commonly found rutaceous species, *Murraya koenigii*. The characteristics of immature and adult stages as well as the sex ratio of emerging adults were investigated.

MATERIALS AND METHODS

Host Plant
Selection of the rutaceous host plants for this study was based on their potential values as commercial plants. Three citrus species, *Citrus reticulata, C. aurantifolia* and *C. hystrix* were selected and a partially domesticated species, *Murraya koenigii* was used for comparison. The citrus seedlings were supplied by Wan Ah Bee Nursery, Balik Pulau, Pulau Pinang while *M. koenigii* seeds were collected from wild plants in Pulau Pinang and planted in the nursery of the School of Biological Sciences, Universiti Sains Malaysia (USM). The young seedlings were transferred into plastic bags (d = 25 cm, h = 35 cm) with podsolic, compost and manure mixture of 3:1:1. They were fertilized fortnightly with a 1/2 teaspoon of artificial fertilizer and 100 g of manure.

Papilio polytes
The life cycle of *P. polytes* consists of egg, larva, pupa and adult. The eggs are yellowish, spherical with a diameter of 1.00–1.08 mm (Salmah *et al.* 2002). Its young larva resembles a bird's dropping but would later moult to become green with a few white or brown markings (Corbet & Pendleburry 1992; Salmah *et al.* 2002). The greenish brown pupa attaches to a stem of the food plant or to a nearby object by a silken girdle (Corbet & Pendleburry 1992). In the adult stage, there are conspicuous tail-like projections in the hind wings of males and females.

The larvae of a *Papilio polytes* (common mormon butterfly) were collected from citrus trees from several areas in Pulau Pinang. They were reared in the laboratory on *Citrus microcarpa*. The larvae were placed in a screen cage (50 cm x 50 cm x 50 cm) and maintained at temperatures of $24^{o}C$–$26^{o}C$, 60%–85% Relative Humidity (RH) and a photoperiod of L12:D12 until emergence. When butterflies emerged, they were transferred into a field cage (2 m x 2 m x 2 m) for mating and oviposition. The eggs laid were collected daily and used in subsequent observations.

Duration of Larval Stages
The eggs were placed in a petri dish (d = 9 cm) layered with a wet tissue paper. A plastic screen with a square fine wire window (2 x 2 cm^2) for ventilation covered the petri dish. The time taken to hatch was recorded. Newly hatched larvae were reared individually in screen cages (50 cm x 50 cm x 50 cm). The first and the second instar larvae were fed with a very young two-leaf citrus twig. The twig was cut from a mother plant and placed in a bottle (d = 1 cm, h = 10 cm) filled with water. As for *M. koenigii*, the larvae were provided with a young twig with four to five leaves. The leaves were substituted daily. Both the duration

of larval stages and body length were recorded daily until the larvae moulted to pupae. The duration of pupal stage on each host plant was also recorded. These observations were replicated thirty times.

Characteristic of Adult
Adult butterflies were killed in ethyl acetate soon after the wings hardened, approximately four hours after emergence. They were dried in an oven at $50^{o}C$ for a week. The length of the adult's body (head to abdomen), antenna and wingspan were measured and recorded. The measurement of the wingspan followed that of Dickson (1976).

Data Analysis
The effect of host plant on life cycle parameters and adult characteristics of *P. polytes* were evaluated using the one-way and two-way analyses of variance (ANOVA). Means associated with each variable of the host plant were further separated using the Tukey's test (Rao 1998). All data were analyzed using the SPSS software version 12.

RESULTS

Immature Stage
Papilio polytes required 28.40 to 29.73 days to complete their cycle from eggs to adults. The eggs hatched after three days. The larval development was completed in 14.63 to 16.13 days. There was a prepupal stage that lasted for a day. The pupal stage took 9.6 to 10.13 days before emerging into adults. The durations of larval and pupal stages were significantly different on different species of host plants ($F_{larva\ 3,.00}$ = 9.887, $F_{pupa\ 3,\ .001}$ = 6.145, $F_{total\ 3,\ .007}$ = 4.25). The shortest immature period (egg until emergence, 28.40 ± 0.25 days) was recorded for the larvae fed with *C. reticulata*. Comparing the durations of immatures (larva and pupa), those on *C. reticulata* were significantly shorter than the larvae feeding on *M. koenigii* (29.73 ± 0.32 days), but not significantly different from the immatures living on the other two citrus species. However, there was no difference in duration of immature stages among *P. polytes* reared on *C. aurantifolia*, *C. hystrix* and *M. koenigii* (Table 1).

Papilio polytes spent the longest larval period on *M. koenigii* (16.13 ± 0.30 days) which was significantly longer compared to other host plants. Among the citrus species, the larval period on *C. reticulata* was the shortest and no difference was detected with *C. aurantifolia* and *C. hystrix*. The pupal period of *P. polytes* on *M. koenigii* (9.60 ± 0.09 days) was significantly shorter than those on *C. hystrix* (10.13 ± 0.09 days), but no significant variation was detected with the larvae on *C. aurantifolia* and *C. reticulata*.

Table 1: Duration of immature stages (mean ± SE) of *P. polytes* reared on different host plants. Mean values in the same column with the same letters are not significantly different at P = 0.05 (Tukey's test). All N = 30.

Host plant	Mean duration of stages (days)		
	Larva	Pupa	Total period
C. aurantifolia	15.30a ± 0.19	9.90ab ± 0.09	29.20ab ± 0.24
C. hystrix	14.73a ± 0.13	10.13b ± 0.09	28.87ab ± 0.17
C. reticulata	14.63a ± 0.22	9.77a ± 0.09	28.40a ± 0.25
M. koenigii	16.13b ± 0.30	9.60a ± 0.09	29.73b ± 0.32

There were five larval instars of *P. polytes*. Except for the fourth instar, the duration of all instar stages that fed on *M. koenigii* were the longest (Table 2). The result of the two-way ANOVA, indicated that the larval duration and the duration of each stadium were significantly different on different host plants ($F_{host\ 3,.000}$ = 11.61, $F_{stadium\ 4,.00}$ = 1056.72, $F_{host\ x\ stadium\ 12,.000}$ = 2.99). The older instars have longer period than the younger instars and the trend of increasing instar durations was approximately similar in all host plants.

Table 2: Duration of larval instars (mean ± SE) on different host plants. Mean values in the same column with the same letters are not significantly different at P = 0.05 (Tukey's test). All N = 30.

Host plant	Duration of larval stadia (days)				
	L 1	L 2	L 3	L 4	L 5
C. aurantifolia	2.03a ± 0.03	2.20a ± 0.07	2.50b ± 0.10	3.17b ± 0.07	5.40a ± 0.11
C. hystrix	2.00a ± 0.00	2.07a ± 0.05	2.07a ± 0.05	2.93ab ± 0.11	5.67ab ± 0.11
C. reticulata	2.00a ± 0.00	2.17a ± 0.07	2.33ab ± 0.09	2.83a ± 0.07	5.30a ± 0.13
M. koenigii	2.07a ± 0.05	2.50b ± 0.09	2.53b ± 0.10	3.07ab ± 0.11	5.97b ± 0.18

Table 3: Body length (mean ± SE) of instars larvae on different host plants. Mean values in the same column with the same letters are not significantly different at P = 0.05 (Tukey's test). All N = 30.

Host plant	Body length (cm)				
	L 1	L 2	L 3	L 4	L 5
C. aurantifolia	0.53b ± 0.01	0.89b ± 0.02	1.54a ± 0.02	2.63a ± 0.03	4.16a ± 0.05
C. hystrix	0.55b ± 0.01	0.98c ± 0.01	1.58a ± 0.03	2.64a ± 0.04	4.23ab ± 0.03
C. reticulata	0.53b ± 0.01	0.88b ± 0.01	1.54a ± 0.03	2.66a ± 0.02	4.31b ± 0.05
M. koenigii	0.49a ± 0.01	0.81a ± 0.02	1.49a ± 0.02	2.65a ± 0.04	4.29ab ± 0.04

Table 4: Length and width (mean $\pm$ SE) of pupa of *P. polytes* on different host plants. Mean values in the same column with the same letters are not significantly different at P = 0.05 (Tukey's test). All N = 30.

Host plant	Length (cm)	Width (cm)
C. aurantifolia	3.04ab $\pm$ 0.02	1.22a $\pm$ 0.01
C. hystrix	3.12b $\pm$ 0.02	1.24a $\pm$ 0.01
C. reticulata	3.05ab $\pm$ 0.05	1.24a $\pm$ 0.04
M. koenigii	2.93a $\pm$ 0.04	1.19a $\pm$ 0.03

Variations in larval sizes were also observed, whereby the body lengths of various larval instars were significantly different among the host plants, with L 1, L 2 and L 3 on *M. koenigii* were the shortest ($F_{host\ 4,.00}$ =10687.5, $F_{host\ x\ body\ length\ 12,.00}$= 4.05) (Table 3). The pupae that emerged from the larvae feeding on citrus host plants were of approximately similar sizes but those fed with *M. koenigii* were significantly shorter than the pupae on *C. hystrix* ($F_{length\ 3,.003}$=4.99, $F_{width\ 3,.53}$ = 0.740) (Table 4).

Characteristics of Adult
The length of body, antenna and wingspan of adults of *P. polytes* varied significantly in various host plants ($F_{body\ length\ 3,.018}$ = 3.475, $F_{antenna\ 3,.009}$ = 4.036, $F_{wingspan\ 3,.038}$ = 2.896). The length of adult's body, antenna and wingspan of the larvae reared on *C. hystrix* were 2.65 $\pm$ 0.13 cm, 1.85 $\pm$ 0.07 cm and 10.03 $\pm$ 0.58 cm respectively. They were significantly longer compared to those fed with *M. koenigii* but no significant variation of the adults on *C. aurantifolia* and *C. reticulata* was detected at P = 0.05. There was no significant variation of adult characteristics measured on butterflies fed with *M. koenigii, C. aurantifolia* and *C. reticulata* (Table 5). The numbers of males and females emerged from different host plants were approximately similar (χ^2_3= 0.1481, P = 0.05) giving a ratio of 1:1.

Table 5: Adult characteristics (mean $\pm$ SE) of *P. polytes* reared on different host plants. Mean values in the same column with the same letters are not significantly different at P = 0.05 (Tukey's test). All N = 30.

Host plant	Adult characteristic (cm)			Sex	
	Body length	Antenna	Wingspan	Male	Female
C. aurantifolia	2.59ab $\pm$ 0.02	1.82ab $\pm$ 0.02	9.69ab $\pm$ 0.07	15	15
C. hystrix	2.65b $\pm$ 0.02	1.85b $\pm$ 0.01	10.03b $\pm$ 0.11	12	18
C. reticulata	2.59ab $\pm$ 0.03	1.79ab $\pm$ 0.02	9.85ab $\pm$ 0.14	16	14
M. koenigii	2.53a $\pm$ 0.02	1.76a $\pm$ 0.02	9.63a $\pm$ 0.09	16	14

DISCUSSIONS

Insect preference on different host plants is governed by the plant nutritional condition. It was found that the water and protein content of the leaf is very important for growth and development of the larvae especially in the early instars (Ojeda-Avila *et al.* 2003). The result of this study showed that the larval period of *P. polytes* fed with *M. koenigii* was longer than those living on *C. aurantifolia, C. hystrix* and *C. reticulata*. The protein content of *M. koenigii* (2.68% protein and 72.04% water) (Suwarno, unpublished data) is much lower than that of many citrus species. For instance, *C. lemontea* contains 4.82% protein and 75.15% water, *C. sinensis has* 4.8% protein and 70.13% water while in *C. hystrix* there is 3.56% protein and 74.15% water (Astuti 1993). When *Papilio demoleus* was reared on *C. lemontea and C. sinensis,* Astuti (1993) observed that the larvae spent 24 days on *C. lemontea and* 26.3 days on *C. sinensis.* Although there was very little variation in protein contents in these two plants, the larvae showed faster growth in the one with higher protein and water content. Similar occurrence was observed in a moth species, *Manduca sexta.* Larval growth was very slow on low water and low protein diets (Wigglessworth 1972; Bernays & Chapman 1994). The amino acids resulting from protein digestion are among the most important raw materials for the growth of the larvae.

Besides higher content of the protein and water in the host plants, variation of the amount of these two components contributes to the differences in the life cycles of phythophagous pests. *Papilio memnon,* another species of Papilionidae, spent 31 days on *C. lemontea* and *C. sinensis* and 39 days on *C. hystrix* (Astuti 1993). In this case, protein seemed to be more important to the larvae than water in the host. The quality of the host plant was also found to influence the time to pupation (Penz & Araujo 1990) and pupal size is positively correlated with water and amino acid contents of the hosts (Scheirs *et al.* 2003). Plant species that supports the fastest growth of the insect is considered the most suitable host.

Other contents of the host plant could also contribute to variations in the rate of growth as well as the size of butterfly pests. While there is no significant difference in the total amount of the foliage consumed by *Pseudoplusia includens* on *Peuraria montana* (Lour.) Merr. (kudzu) and *Glycine max* (L.) Merr. (soybean), the size of larvae that fed on *G. max* were twice as large as those fed on kudzu (Kidd & Orr 2001). The larval durations were also significantly shorter. The crude protein content of soybean and kudzu were 24.7% and 23.7% respectively and the dry matter content was much higher for soybean (25%) than kudzu (22.5%). Large difference in the dry matter content could account for larger larval size and shorter duration of larval stage on soybean.

The larval duration and length of the fifth instar *P. polytes* on all host plants were longer than the first, second, third and fourth instars. Usually, during the fifth instar the larva eats as much as 80% of total food intake of all larval instars. The size of the larva increases proportionately to the amount of food consumed. Limited availability of food will result in small adults although the diet is nutritionally sufficient (Chapman 1975). Nutrient reserves in the larva are used for formation of various organs during pupal stage for major internal

reconstructions that occur during this stage. Presumably, its greatest importance is for permitting the full development of wings and flight muscles (Chapman 1975). The pupal size is very much dependent on the size of the fifth instar larva which eventually determines the size of adult. Response of larvae to host quality may involve compromises between size and time of emergence of adults, or gathering of resources for reproduction by larval and adult stages (Singer 1984). A larva that finds itself on a poor host may either lengthen its generation time to become an adult of normal size or conserve its larval duration and become a smaller adult with little reserves.

Among all the *Citrus* species examined, the duration of fifth instar larvae on *C. hystrix* was longer than on *C. aurantifolia* and *C. reticulata* and the pupal size was the biggest. The size of pupae determined the size of adults since the adults that emerged from immatures reared on *C. hystrix* were the biggest. Examining nutritional physiology of different sexes of caterpillar separately, Mackey (1978) and Lederhouse *et al.* (1982) concluded that females achieve their greater sizes by feeding and developing for a longer period of times. Throughout most of the life cycle in Lepidoptera, females tend to be heavier than males, a feature that has been attributed to the role of producing eggs (Scriber & Slansky 1981). Even though the ratio was not statistically significant, it was interesting to note that more females were produced from *C. hystrix* (Table 5).

This study revealed that *C. reticulata* and *C. hystrix* were the most suitable among the four hosts for this butterfly. The shortest larval duration and subsequently the life cycle duration was recorded from *C. reticulata* whereas the biggest pupae and adults occurred on *C. hystrix*. *Murraya koenigii* was the least preferred. Arranged in decreasing order of preference, the four hosts are *C. reticulate*, *C. hystrix*, *C. aurantifolia and M. koenigii.* As a general conclusion, all citrus species were suitable hosts for *P. polytes* whereby *M. koenigii* was the least preferred among the four hosts examined. In the absence of citrus plants, *M. koenigii* would serve as an alternative host for this species.

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