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# Preliminary Study of Semi-synthetic Artificial Diet for the *Idea leuconoe clara* (Butler) (Lepidoptera:Nymphalidae)

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## Abstract

In this paper, We emphasized on the research and development of the semi-synthetic artificial diet for the giant danaine butterfly, *Idea leuconoe clara* (Butler). Our experiment involved feeding the larvae leaves of host plant, Helicoid-stamenal Parsonia, *Parsonia laevigata* (Moon) Alston, as control group, and fed the artificial diet as experimental group, In order to study the feasibility of rearing this species with the artificial diet, we reared giant danaine butterfly individually and kept them in the growth chamber with a temperature of 25±1℃, a humidity of 80±5 % RH, and a photoperiod of 14L: 10D as the controlled conditions. Hatching larvae were individually reared with either the semi-synthetic artificial diet or the leaves of Helicoid-stamenal Parsonia until adult emergence. The survival rate of the experimental group, from the first instar larvae of new hatching to became adults, was 58.3%, which was less than the control group 78.3%. The average developmental period of the experimental group, from the first instar larvae of new hatching to became adults, was 10.7 days longer than those of the control group - that is, 47.15 ± 0.29 and 36.40 ± 0.28 days, respectively. This butterfly species, reared with the artificial diet, had a lower survival rate during every life stage and had a longer developmental period. The width of the head capsule in the experimental group from those of the first instar to the fourth instar larvae was smaller than those of the control group, and the differences between the various instars from the two groups were all significant. However, the head capsule's width of the two groups, in the fifth instar larvae, was the same. Therefore, the threshold value of head capsule's width for the larva's development to pupa was above 4.11 mm. The increment of the head capsule's width of the larva was in accordance with the increase in the larval instar stage; it maintained a ratio of 1.5 times. The experiment with this species accorded with Dyar's law. Though up to 60% of the adult wings from the experimental group were creased and unable to fly normally, the appearance and wing color of both groups had no difference. Those adults, from the experimental group, that had normal wings could fly normally. They had mating and reproductive capability. Though some of the biological characteristics of the giant danaine butterfly were lower than those of the control group, the experiment reared adults, from the experimental group, that had a survival rate of over 58.3%. As a result, rearing the giant danaine butterfly with the artificial diet is feasible, but the diet still has some shortcomings that need to be studied and improved in the future.

**Keywords**: giant danaine butterfly, *Idea leuconoe clara*, semi-synthetic artificial diet, Helicoid-stamenal Parsonia, *Parsonia laevigata*, rear, biological characters.

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# 大白斑蝶(*Idea leuconoe clara* (Butler)) (鱗翅目：蛺蝶科)的半合成人工飼料初探

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## 摘　要

本研究在研發大白斑蝶(*Idea leuconoe clara* (Butler))的半合成人工飼料，並以天然食草爬森藤(*Parsonia laevigata* (Moon) Alston)葉片飼育爲對照組，探討此人工飼料飼育本蝶種的可行性。試驗採用單隻飼育的方式，置於 25 ± 1 ℃、80 ± 5% RH、14 L：10 D 條件的生長箱內，分別以半合成人工飼料及天然食草爬森藤葉片，餵飼剛孵化的幼蟲至羽化爲成蝶。由試驗結果顯示，剛孵化的一齡幼蟲生長發育至羽化成蝶的存活率，飼料組爲 58.3 %較葉片飼養的 78.3 % 低；平均發育日數，飼料組爲 47.15 ± 0.29 日，較葉片組的 36.40 ± 0.28 日長約 10.7 日，因此，以人工飼料飼育的各蟲期存活率較低且所需發育日數較長。第一至第四齡幼蟲的頭殼寬度，飼料組皆小於葉片組，且兩組間皆有顯著性

差異，但至第五齡幼蟲兩組間則幾乎相同，可知本蝶種幼蟲頭殼寬度超過 4.11 mm 的閥值，即可進入化蛹階段。兩組的幼蟲頭殼寬度皆隨齡期以約 1.5 倍等比增加，符合戴爾法則。雖然飼料組有高達 60%成蟲翅膀皺縮，無法正常飛翔，但在外型及翅膀顏色方面，兩組並無差異，而人工飼料組中翅膀正常的成蝶均可正常飛翔、交尾和繁殖後代的能力。由此可知，本試驗以半合成人工飼料餵飼大白斑蝶的試驗結果，顯示飼料組仍有一些生物特性表現不如葉片組，但飼養之幼蟲至成蝶存活率可達 58.3%，故用此半合成人工飼料飼育本蝶種確實可行，唯此人工飼料的配方仍有些缺點，需待日後再研究改進。

**關鍵詞**：大白斑蝶、半合成人工飼料、爬森藤、飼育、生物特性

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## Introduction

Research and development of artificial diets for insects have a century of history in Taiwan and other countries. Singh (1977) reported about the research and development of the composition of artificial diet, diet preparation, and the process for rearing insect for 10 orders. This included over 700 insect species since 1908, but only two or three butterfly species can be reared on artificial diets. Therefore, it is generally not as easy to rear butterflies on artificial diets. According to Holloway *et al*. (1991), there could be several reasons for this. For example, many moth species are generalists with respect to the host plant whilst butterflies tend to be more specialized. Also, some moths are capable of boring themselves into solid materials, a behavior that butterflies, on the whole, do not possess. Although butterflies are frequently used as experimental material, they are usually fed with living plants in their larval stages. This is expensive both in terms of space and time (Holloway *et al*., 1991). However, artificial diets are useful for the mass rearing of phytophagous insects in the laboratory. Development of artificial diets containing less dried leaf powder would be important when the insect species' host plant is rare or when the larvae consume a large amount of leaves (Yoshio and Ishii, 1996).

Gnec and Nation (2004) thought the need to study and control insect pests has probably contributed to the development of artificial diets for many insects, but most butterflies are not pests on economic crops and little effort has been devoted to developing artificial rearing media for them. Butterflies tend to be restricted to one or only a few host plants as larvae, and they are possibly very sensitive to the balance of nutrients and/or presence of specific feeding cues in their host plants. Therefore, there were fewer pieces of literatures pertaining to the artificial diet of butterflies than the other insects until now, especially in the research of Nymphalidae Danainae. The monarch butterfly, *Danaus plexippus* (Linnaeus), also called Monarque, is a better-known butterfly outside of Taiwan; Singh and Clare (1988) have already developed its artificial diet and rearing method in New Zealand. After many years, Leong (2001) researched and developed the semi-artificial diet of the monarch butterfly, and he successfully owned the United States Patent. Therefore, we can buy commercialized artificial monarch butterfly larval diets in the market. However, Luo (1988) was the only person to rear the plain tiger (*Danaus chrysippus* (Linnaeus), the thesis used *Anosia chrysippus* at that time) with the artificial diet for many years in Taiwan. There is also no commercialized and specialized artificial diet for Danainae larvae in Taiwan.

The giant danaine butterfly, *Idea leuconoe clara* (Butler), is the biggest among the Danainae butterflies in Taiwan. Their common names are Rice paper butterfly, Paper kite butterfly, Wood nymph, and Siam tree nymph. This butterfly species is easy to observe because of its marked wing mottle and slower motion. It is one of the most important, spectacular butterfly species in Kenting National Park (Tsai, 1992). Because the giant danaine butterfly is appreciated for its ornamental value and is easy to observe, it can be used as field educational materials. In recent years, Taiwan government promoted recreational visits and ecological tours, which created many leisure vacation farms and butterfly gardens for sightseeing. Since the giant danaine butterfly is not shy of humans, and it flies in a carefree manner, leisurely, and slowly, making it one of the most attractive butterfly species. But the artificial rearing tends to be less successful, due to the feeding problem brought about by the larvae consuming only the leaves of host plant.

Giant danaine butterfly larvae feed exclusively on *Parsonia laevigata* Alston (Apocynaceae) in the Ryukyus (Nishida *et al*., 1996). Our observation pointed out that the larvae feed exclusively on Helicoid-stamenal Parsonia, *Parsonsia laevigata* (Moon) Alston, in Taiwan, and no other host plant has been reported. Therefore, it can be concluded that the giant danaine butterfly is a monophagous insect in Taiwan. However, Helicoid-stamenal Parsonia, which is difficult to obtain or buy, is a wild plant and not a horticultural crop. According to Chen *et al*. (2003), rearing this butterfly species from an egg to a pupa required a preparation of at least 40 to 60 leaves per butterfly. In other words, every giant danaine butterfly larva consumes all the leaves per host plant to finish its growth and development. In the long run, processing the host plant into dry powder that can be kept for a long time while researching and developing the formula for the semi-synthetic artificial diet can help mass produce this species of butterfly. As a result, we developed the semi-synthetic artificial diet of giant danaine butterfly, and studied the feasibility of rearing this species on the artificial diet.

## Materials and Methods

1. Composition of the semi-synthetic artificial diet and its preparation

We plucked the leaves of the Helicoid-stamenal Parsonia washed them, and had them freeze dried. They were then ground its to a very fine powder with a mill, stored, and refrigerated until used. The composition of our semi-synthetic artificial diet for the giant danaine butterfly is the one modified from Mattoni *et al*. (2003) and Genc and Nation (2004). We then conducted pretest to improve the formula (App. Table 1).

A mill is used to grind common beans into powder, which is then mixed with dried Helicoid-stamenal Parsonia leaf powder, wheat germ, yeast, casein, gelcarin, methyl paraben, sorbic acid, Wesson salt mix, choline chloride, sucrose, and glucose in a cooking pot. The mixture is then dissolved in the distilled water, heated slowly on the induction cooker, and thoroughly stirred with a glass rod stir in order to disperse the material evenly. After doing so, the induction cooker is switched off. Formaldehyde, vitamin, and ascorbic acid were then added into the mixture and stirred. Finally, tetracycline was added and mixed thoroughly. This mixture is poured into a rectangular fresh keeping box (9.5 cm in length × 7 cm in width × 2.8 cm in height), allowing it to cool and solidify at room temperature. The diet that was made then stored in a refrigerator until use.

2. The butterfly and general rearing method

About 50 adult giant danaine butterflies (♀=20, ♂=30) were collected from the Department of Plant Protection, National Pingtung University of Science and Technology in southern Taiwan. They were carried back and released into a simple net room to breed for generations. They were released into the simple net room and lived there until the start of the experiment. The net room was constructed with galvanized iron pipes and covered with a single Paichi net (16×16 mesh, 6.1 m × 4.7 m × 2.6 m). The larval food source is the plant, Helicoids-stamenal Parsonia , *P. laevigata*, adult honey plants, Common Lantana, *Lantana camara* L., Starcluster , *Pentas lanceolata* Deflerss, Blood Flower, *Asclepias curassavica* L., African Touch-me-not, *Impatiens wallerana* Hook. f., White Champaka, *Michelia alba* D. C., and Jamaica False-valarian, *Stachytarpheta jamaicensis* (L.) Vahl. These were all planted in the net room. Adult giant danaine butterfly can use these plants to suck nectar, initiate their calling behavior, copulate, and hide from the sun and rain. These plants also provide places for the adults female to oviposit and food for larvae to feed on. After the giant danaine butterfly reproduced for three to four generations in the net room, the experiment and observation started.

3. Experimental method

Before the experiment, pots of Helicoids-stamenal Parsonia were planted outside the net room. When they grew up to about 100 cm in height and had about 40 leaves, two bonsais (Helicoids-stamenal Parsonia) were carried into the net room so that the female butterflies could use them to oviposit. We removed the bonsais plant from the net room the next day to check on eggs oviposited on the leaves and we found the eggs to bring back the laboratory. Each petiole was wrapped with cotton cloth stripes and dripped with water to keep it wet. Then, each leaf holding the egg was placed into a rectangle plastic box (25 cm × 18.5 cm × 9 cm). The box was stored in the growth chamber, which was set up on a 25±1℃, 80±5 % RH, 14L : 10D (lights on at 5:00 and off at 19:00) constant condition. When the eggs hatched, we divided the 120 newly hatched larvae into two groups. One group, which was reared individually with the artificial diet, was placed into a small round transparent plastic box (4.0 cm in diameter at the mouth, 3.0 cm in diameter at the base, 2.5 cm in height, and about 20 ml in capacity). The other group, which was reared with just the matured Helicoid-stamenal Parsonia leaves, was put into a round transparent plastic box (9.2 cm in diameter at the mouth, 8.0 cm in diameter at the base, 5.8 cm in height, about 300 ml in capacity). Each box containing its larval group was cleaned every day. Both groups were fed their respective diets. Once the larvae hit the third instar stage, the original containers were replaced with a larger opaque plastic box (11.0 cm in diameter at the mouth, 9.0 cm in diameter at the base, 8.0 cm in height, about 500 ml in capacity). On the cover of each box was a punctured hole that was covered with a 2 × 2 cm screen (36×36 mesh) for ventilation. The growth, development, and morphological change of the Giant danaine butterfly were visually observed and recorded every day. Their body length and head capsule width of larvae were also measured.

The head capsule width was measured using head capsules of exuviae at the time of ecdysis. The head capsule width of the first instar larvae was calculated using the ocular micrometer under the anatomic microscope. The head capsule width of other instar larvae was also measured with the digimatic caliper. The body length of the first instar larvae that hatched and the other instar larvae after ecdysis was measured with the digimatic caliper. The pupa weight was measured with an electronic balance. After the larvae pupated, we stuck and hung a few strips of silk socks or toilet papers into the rearing plastic box to help the butterflies emerge. The wingspan and the width of the adults after eclosion were measured with the digimatic caliper. The weight of adults was measured with an electronic balance. We then compared the significantly different of means by using the *t*-test.

## Results

1. Survival rate and developmental period

The results involving the survival rate of the giant danaine butterflies that were reared with the semi-synthetic artificial diet and the leaves of the host plant, Helicoid-stamenal Parsonia, are listed in Table 1. The lowest survival rate of larva reared with semi-synthetic artificial diet was that of the first instar

larvae, with only an 83.3% survival rate. The survival rates of the other instar larvae were all over 91.0%. Comparing the larval survival rates of both reared groups, the survival rate of the fifth instar larvae in the control group was only slightly less than that of the experimental group. The other instar larvae stages all had a higher survival rate than the larvae of the experimental group. The results as a whole larval stage showed that the survival rate of the experimental group was only 72.4%, which was about 15.9% less than the control group's 88.3%. The survival rate of the pupa for the control group was 88.7%, which was higher than the experimental group's 81.4%. The survival rate of the experimental group, from the first instar of new hatching to became adults, was 58.3%, which is 20.0% less than the control group's 78.3%.

Table 1 Survival rate of various developmental stages of *Idea leuconoe clara* reared with semi-synthetic artificial diet and leaves of host plant

| Life stage | Survival rate (%) (*n*)[1] | |
|---|---|---|
| | Artificial diet | Leaves of Helicoid-stamenal Parsonia |
| 1st instar larva | 83.3 (50) | 93.3 (56) |
| 2nd instar larva | 98.0 (49) | 100.0 (56) |
| 3rd instar larva | 98.0 (48) | 100.0 (56) |
| 4th instar larva | 91.7 (44) | 98.2 (55) |
| 5th instar larva | 97.7 (43) | 96.4 (53) |
| Larvae | 72.4 (43) | 88.3 (53) |
| Pupa | 81.4 (35) | 88.7 (47) |
| Larva to Adult | 58.3 (35) | 78.3 (47) |

1) *n* in parentheses is the number of larvae observed

In this experiment, all the pupae in both groups could eclosion to adults. Out of 60 new hatching larvae in the experimental group, only 35 pupae could live to become adults. In the control group, there were 47 adult emergences, including 25 males and 22 females. In the experimental group, there were 35 adult eclosion, including 18 females and 17 males. However, out of the 35 adults, there were 21 that failed to expand their wings. Those with these aberrant wings, which were creased, were unable to fly normally and were considered crippled. The remaining 14 adults (3 males; 11 females) were normal and all had the ability to mate and reproduce.

The average developmental period from the first to fifth instar larval stages of the experimental group were 4.35 ± 0.11, 4.19 ± 0.09, 5.32 ± 0.15, 6.37 ± 0.24, 11.48 ± 0.18 days, respectively (Table 2). These durations were longer than those of the control group ($p < 0.01$). The developmental period of the larval stage for both groups were 31.38 ± 0.38 and 20.87 ± 0.30 days, indicating that the larvae of experimental group developed 10.5 days slower than the control group ($p < 0.01$). The developmental period of the pupa was 16.50 ± 0.21 days for the experimental group and 15.83 ± 0.08 days for the control group. Though the difference was only about one day, using the *t*-test showed that a significant difference still existed between them ($p < 0.05$) . The average developmental period of the experimental group, from the larvae hatched to their adult form, was 10.7 days longer than that those of the control group. For the experimental group, the number was 47.15 ± 0.29, and for the control group, it was 36.40 ± 0.28 days ($p < 0.01$). Based on the result, giant danaine butterflies reared with the kind of artificial diet researched and developed for this experiment had a lower survival rate and longer developmental period in every life stage, compared with those reared with Helicoid-stamenal Parsonia.

Table 2 Duration in days of various developmental stages of *Idea leuconoe clara* reared with semi-synthetic artificial diet and leaves of host plant

| Life stage | Developmental period (days) ($n$)[1] | | | | |
|---|---|---|---|---|---|
| | Artificial diet | | Leaves of Helicoid-stamenal Parsonia | | t-value[2] |
| | Mean±SE | Range | Mean±SE | Range | |
| 1st instar larva | 4.35 ± 0.11 (50) | 3 - 7 | 3.02 ± 0.07 (56) | 2 - 5 | 10.39** |
| 2nd instar larva | 4.19 ± 0.09 (49) | 3 - 5 | 2.63 ± 0.08 (56) | 2 - 4 | 13.29** |
| 3rd instar larva | 5.32 ± 0.15 (48) | 4 - 8 | 3.05 ± 0.04 (56) | 3 - 4 | 15.80** |
| 4th instar larva | 6.37 ± 0.24 (44) | 4 - 12 | 4.36 ± 0.12 (55) | 4 - 9 | 7.93** |
| 5th instar larva | 11.48 ± 0.18 (43) | 10 - 14 | 7.87 ± 0.17 (53) | 6 - 12 | 14.63** |
| Larval stage | 31.38 ± 0.38 (43) | 28 - 38 | 20.87 ± 0.30 (53) | 18 - 30 | 22.26** |
| Pupa | 16.50 ± 0.21 (35) | 15 - 21 | 15.83 ± 0.08 (47) | 14 - 17 | 3.28* |
| Larva to Adult | 47.15 ± 0.29 (35) | 46 - 54 | 36.40 ± 0.28 (47) | 34 - 44 | 26.32** |

1) *n* in parentheses is the number of larvae observed.
2) One or two asterisk in t-value indicate significantly different at 95% or 99% confidence level ($p < 0.05$ or $p < 0.01$), by t-test.

2. Head capsule width and body length in larval stage

The head capsule width of various developmental stages of the giant danaine butterflies of both groups is listed in Table 3. The head capsule width of the larvae of both groups increased, with a similar growth pattern, as the larval instar grew. The head capsule width of the first instar to the fifth instar larvae of the experimental group was 0.75 ± 0.01, 1.17 ± 0.01, 1.74 ± 0.01, 2.50 ± 0.02, 4.11 ± 0.03 mm. For the control group, the calculations were 0.81 ± 0.01, 1.25 ± 0.01, 1.93 ± 0.01, 2.86 ± 0.01, 4.11 ± 0.02 mm ($p < 0.01$). These results mean that the head capsule width of each instar larvae for the experimental group is less than or equal to that of the control group. However there was no significant difference between the widths of the fifth instar larvae from both groups. The data of the two groups are almost in accordance, so we believe the threshold value of the head capsule width from the larva development to pupa was above 4.11 mm. The head capsule width of the giant danaine butterfly larvae had a certain width in accordance to the larval instar.

Table 3 The head capsule width of each instar larvae of *Idea leuconoe clara* reared with semi-synthetic artificial diet and leaves of host plant

| Larval stage | Head capsule width (Mean ± SE, mm) (*n*)[1)] | | |
|---|---|---|---|
| | Artificial diet | Leaves of Helicoid-stamenal Parsonia | t-value[2)] |
| First instar | 0.75 ± 0.01 (50) | 0.81 ± 0.01 (56) | 8.62** |
| Second instar | 1.17 ± 0.01 (49) | 1.25 ± 0.01 (56) | 6.24** |
| Third instar | 1.74 ± 0.01 (48) | 1.93 ± 0.01 (56) | 11.87** |
| Fourth instar | 2.50 ± 0.02 (44) | 2.86 ± 0.01 (56) | 16.30** |
| Fifth instar | 4.11 ± 0.03 (38)[3)] | 4.11 ± 0.02 (53) | 0.16 |

1) *n* in parentheses is the number of larvae observed.
2) Two asterisk in t-value indicates significantly different at 99% confidence level ($p < 0.01$), by t-test.
3) The number of the larvae observed should be 43, but because 5 data records were missing, we could only record that 38 larvae were observed.

For the experimental group, as the larval instar stage increased, the head capsule width also increased from 1.44 to 1.64 times, mean 1.53±0.09 times. For the control group, the increase had a ratio of 1.43~1.54 times, with the average of 1.50 ± 0.05 times. The increment of the head capsule width of the larva was in accordance with the increase in the larval instar stage and maintained a ratio of 1.5 times in both groups (Table 4). This species followed Dyar's law. In other word, it does not matter if the larvae are reared with the artificial diet or the leaves of Helicoid-stamenal Parsonia. The common logarithms of the head capsule width of the giant danaine butterfly larva had a direct correlation with the increase of the larval instar stage and maintained a linear growth pattern.

On the other hand, the body length of the larvae of the two groups increased, with a similar growth pattern, as the larval instar grew (Table 5). The body length of the first instar to the fifth instar larvae for experimental group were 4.63 ± 0.08, 7.29 ± 0.10, 12.92 ± 0.16, 20.59 ± 0.31, 32.48 ± 0.41 mm respectively. The body length for the same categories of larvae belonging to the control group were 4.47 ± 0.07, 8.37 ± 0.01, 12.55 ± 0.14, 20.46 ± 0.23, 31.16 ± 0.28 mm respectively. We found the body length of the second instar larvae to have a significantly difference ($p < 0.05$). We also found that there was no significant difference between the groups of the other instar stage larvae. The body length of each instar larvae for the experimental group was slightly higher than that of the control group. There was not much influence toward the body length of the larvae of this species when it was reared on the artificial diet.

Table 4 The average ratio of head capsule width of the successive instar larvae divided by that of the previous instar larvae to obtain, and the larvae of *Idea leuconoe clara* reared with semi-artificial diet and leaves of host plant

| Larval stage | Semi-artificial diet | Leaves of Helicoid-stamenal Parsonia |
|---|---|---|
| 2nd/1st | 1.56 | 1.54 |
| 3rd/2nd | 1.49 | 1.54 |
| 4th/3rd | 1.44 | 1.48 |
| 5th/4th | 1.64 | 1.43 |
| Average | 1.53±0.09 | 1.50±0.05 |

Table 5 The body length of each instar larvae of *Idea leuconoe clara* reared with semi-synthetic artificial diet and leaves of host plant

| Larval stage | Body length (Mean ± SE, mm) ($n$)[1)] | | |
|---|---|---|---|
| | Artificial diet | Leaves of Helicoid-stamenal Parsonia | t-value[2)] |
| First instar | 4.63 ± 0.08 (60) | 4.47 ± 0.07 (60) | 1.52 |
| Second instar | 7.29 ± 0.10 (50) | 8.37 ± 0.01 (56) | 8.02* |
| Third instar | 12.92 ± 0.16 (48) | 12.55 ± 0.14 (56) | 1.76 |
| Fourth instar | 20.59 ± 0.31 (47) | 20.46 ± 0.23 (56) | 0.36 |
| Fifth instar | 32.48 ± 0.41 (43) | 31.16 ± 0.28 (56) | 2.75 |

1) $n$ in parentheses is the number of larvae observed.
2) Asterisk in t-value indicates significantly different at 95% confidence level ($p < 0.05$), by t-test.

3. The weight of pupa and adult wing length, width and weight

The weight of pupa for experimental group was 1.83 ± 0.04 g. For the control group, it was 1.74 ± 0.03 g (Table 6). Although the former is slightly heavier than the latter, they did not have significant difference between them. Some of the adults from the experimental group that had eclosed had creased wings, but all of the adults in the control group were normal ($n$ = 47). Therefore, we only measured the wing length and width of normal butterflies. The wing length of the adults from the experimental group was 61.68 ± 1.08 mm, slightly shorter than the control group's 64.57 ± 0.34 mm ($p < 0.05$). However, the wing widths of the adults for both groups were all between 43.66 to 43.97 mm, showing no significant difference. The weight of the adults in the experimental group was 0.68 ± 0.15 g, slightly heavier than the control group's 0.59 ± 0.01 g ($p < 0.05$). The wing's length of an adult was shorter and the adult's weight was slightly heavier for the experimental group as compared to the control group. In terms of appearance and wing color, both groups did not have any differences. All normal winged adults could fly normally in the experimental group. These adults all had the ability to mate and reproduce.

Table 6 The weight of pupa, the length and width of the adult wing, and the weight of *Idea leuconoe clara* reared with semi-synthetic artificial diet and leaves of host plant

| Items | Mean ± SE ($n$)[1] | | |
|---|---|---|---|
| | Artificial diet | Leaves of Helicoid-stamenal Parsonia | t-value[2] |
| Pupal weight (g) | 1.83 ± 0.04 (35) | 1.74 ± 0.03 (48) | 1.75 |
| Wing length (mm) | 61.68 ± 1.08 (14) | 64.57 ± 0.34 (47) | 3.41* |
| Wing width (mm) | 43.66 ± 0.90 (14) | 43.97 ± 0.30 (47) | 0.42 |
| Adult weight (g) | 0.68 ± 0.15 (35) | 0.59 ± 0.01 (48) | 3.68* |

1) $n$ in parentheses is the number of samples observed.
2) Asterisk in t-value indicates significantly different at 95% confidence level ($p < 0.05$), by t-test.

## Discussion

The first instar larvae had the lowest survival rate for both groups regardless of diet. The survival rate of the first instar larva for both groups was 83.3% and 93.3% (Table 1), indicating that the experimental group's survival rate was 10.0% lower than that of the control group. Because the survival rate of the control group in this experiment was close to 92.5% - the figure Chen *et al*. (2003) had obtained under a similar condition - we speculate that some of the first instar larvae may have died because they were not able to adapt to the physical conditions of the artificial diet, such as humidity, hardness, coarse degree, and surface form, which influence feeding and digesting. We also felt that chemical factors like phagostimulants or feeding deterrents of the artificial diet may also affect insect feeding behaviors, making these larvae unable to settle down on the artificial diet. Our thinking is similar to Yoshio and Ishii's (1996) test on the great mormon butterfly, *Papilio memnon* L., that was reared with 5 types of artificial diets. They mentioned most of the first instar larvae died from the fact that they were unable to settle down on the two artificial diets. From the hatching of the first instar larva to its adult form, the survival rate of the larvae of the control group was 78.3%. According to Chen *et al*. (2003), the survival rate from a fresh egg to an adult was 51.0%. Note that this statistic was based on 80.0% of the eggs hatching. When the rate was converted to the survival rate, it was 63.8%, of the first instar larva that hatched to adult form, 14.5% less than that of our experiment. The survival rate of the experimental group was 58.3% in our experiment, 20% less than that of the control group, and slightly lower than the result from Chen *et al*. (2003).

The developmental period of the giant danaine butterfly from an egg to an adult was 39.67 days in Chen *et al*. (2003). If 5.30 days is deducted from the egg stage, making it 34.37 days, the developmental period is 2 days less than the control group's developmental period of 36.40 days. That is 12.78 days shorter than the experimental group's developmental period of 47.15 days. The developmental period of the experimental group is much slower than the control group. The difference that was most noticeable was that of the developmental period regarding the fifth instar larvae of both groups. The developmental period for the experimental and control groups were 11.48 days and 7.87 days, respectively.

The head capsule width of the first instar to the fourth instar larvae belonging to the experimental group was smaller than that of the control group in our experiment. However, the head capsule width of the fifth instar larvae of both groups were the same as Chen *et al*. (2003), which was 4.11 mm. Therefore, the threshold value of the head capsule width for the larva development to pupa was above 4.11 mm. The body length of each instar larvae in the experimental group was higher than that of the control group in our experiment. The body length of the fifth instar larvae of experimental and control groups were 32.48 mm and 31.16 mm respectively, calculations that are similar to Chen's *et al.* (2003), which was 31.44 mm. The survival rate of every life stage was lower and the developmental period was longer for experimental group. However, while the larvae grew into the fifth instar, the fact that they were reared with the artificial diet or the leaves had little influence. No matter how much time it took to develop, the head capsule width and body length of the fifth instar larvae must reach a certain threshold before it could pupate.

For this species, the weight of the pupa was 1.75 g in Chen *et al*. (2003), similar to the 1.74 g that we calculated for the control group in our experiment. Both of these were lighter than the experimental group, which had a weight of 1.83 g, but did not show significant difference. The wing's length of the adult for the control group was 64.57 mm, which was longer than the experimental group's 61.68 mm, and the latter was similar to Chen's *et al.* (2003) 61.67 mm. The width of the adult wings were 43.97 mm and 43.66 mm for control and experimental groups in this experiment, respectively, both of which were also larger than the 41.80 mm that Chen *et al*. (2003) calculated. The weight of the adult in the control group was 0.59 g, and 0.68 g in the experimental group, showing that the control group was slightly lighter. Therefore, the weight of the pupa and wing's width of the adult in the experimental group was similar to that of the control group. However, the wing length and weight of the adult in the experimental group were slightly shorter and heavier than that of the control group. In addition, the morphology and wing color of the adult for the experimental group was the same as those of the control group, and all adults belonging to the experimental group with normal wings could fly normally. They had mating and reproductive capability.

Though the giant danaine butterfly is appreciated for its ornamental value and is easy to observe, its natural host plant, the Helicoid-stamenal Parsonia, being a wild plant and difficult to obtain, making mass rearing a difficult problem. On the other hand, artificial diet is a quality homogeneous, at all seasons food source. In fact, the ideal artificial diet formula for the insect would be the one that does not include the host plant powder at all, and it is suitable for many insect species, allowing them to grow, develop, reproduce, and complete their life cycles normally. As Yoshio and Ishii (1996) had mentioned, the development of artificial diets containing less dried leaf powder would be important for rearing such a species when its host plant is rare or when the larvae consume a large amount of leaves. The research of the moth, Lepidoptera, has already achieved this. For example, according to the composition published by Ou-Yang and Chu (1988), they started preparing their own artificial diet composition since 1984, and they reared *Spodoptera litura* (Fabricius) and bred it for generations in order to carry out a series of tests in the laboratory. However, there were difficulties with the butterflies. Genc and Nation (2004) studied the artificial diet composition for the Nymphalidae, phaon crescent butterfly, *Phyciodes phaon* (Edwards). The result showed that although the ovaries of females produced on host-free artificial diet appeared to be mature at emergence and contained mature-looking eggs, we never obtained viable eggs from them. The small amount of host leaves in the artificial diet may aid digestion and assimilation of nutrients. The host plant's tissue plays a critical role in the nutrition and reproduction faculties of both the male and female phaon crescents.

How do we make the insects consume the artificial diet that is totally different from their host plants? Genc (2006) thought phagostimulants may be nutritional components or nonnutritional allelochemicals. Most food chemical components stimulate feeding in one insect or another, but if a particular chemical cue that an insect needs is missing, the insect may reject food that would otherwise be considered nutritionally sufficient. Morton (1981) also stated that most herbivorous species require a phagostimulant to bring about feed behavior. This is often some component peculiar to the food plant. Phagostimulant is usually obtained by adding dried food plant material at about 1.5-2.0% w/w. In some reports over 40% of plant material has been combined in the diet (Morton, 1981).

In most cases, the research and development of the artificial diet for the butterfly will not succeed unless it contains a composition of the host plant. For example, Genc and Nation (2004) mentioned the PB diet designed for moths could be used for rearing phaon crescent butterfly. Using such a method allowed only about 37% of newly hatched larvae to become adults, and these adults did not reproduce. The survival rate up to the adult stage was raised to 66% by adding to the diet (10% by weight) freeze-dried leaves of the host plant, *Phyla nodiflora* (L.), and as a result, four successive generations were produced. Singh and Clare (1988) reported the artificial diet composition of the monarch butterfly, *Danaus plexippus* (Linnaeus). It contained 13.9% freeze-dried Swan Plant, *Asclepias fruticosa* (Linnaeus), leaf powder. Yoshio and Ishii (1996) reared the great mormon butterfly with 5 types of artificial diets containing a small amount (7-9% by weight) of dried leaf powder containing *Citrus* sp. and /or *Zanthoxylum ailanthoides* (Rutaceae). The artificial diet of the giant danaine butterfly that was researched and developed in our experiment only contained 2.57 % Helicoid-stamenal Parsonia leaf powder, a substantially reduced proportion of host plant.

Although the larvae reared with the artificial diet had a total of 35 adult eclosions in this experiment, 21 adults had creased wings and were unable to fly normally. Mattoni *et al*. (2003), who reared the palos verdes blue butterfly, *Glaucopsyche lygdamus palosverdesensis*, also stated that the adults were unable to molt the pupal shells or failed to expand their wings. Even those that reached adult stage and were reared with the artificial diet had defective legs and wing aberrations. However, Holloway *et al*. (1991) reported that when they reared *Bicyclus* spp. butterflies with the artificial diet, they often resulted in survivals of over 90%, sometimes even over 95% in the earlier trials, but extensive crippling of the wings were also noted in all of these earlier trials. Indeed, it is generally believed in those works that the butterflies may have been unable to find certain essential fatty acids in the diets designed for moths. Thus, the addition of linseed oil to the original diet was attempted; as a result, they successfully settled the problem and reared *B. anynana*, *B. safitza* and *B. ena*. Therefore, wings folds of giant danaine butterfly reared with the artificial diet in our experiment may also result from a similar reason.

Singh and Clare (1988) researched and developed the artificial diet and rearing method for the monarch butterfly in New Zealand. Although the fecundity data was not obtained due to the small number of specimens reared, the butterflies that emerged were comparable in size and color to field collected specimens. Over 10 years later, Leong (2001) was successfully able to obtain the patent and developed the artificial diet, and as a result, became a commercial goods selling in the United States of America. However, research and development of the semi-synthetic artificial diet for the giant danaine butterfly in this experiment, though some of the biological characteristics in the experimental group were not as good as that in the control group, already reared adults with a survival rate of over 58.3 %, that were comparable in appearance and color to that of adults in the control group, and that had mated and reproduced capability. Although shortcomings did exist, we consider it is a breakthrough. Just like Genc (2006) stated, although basic nutritional needs of insects are well understood, developing suitable diets can still be very difficult. Currently, research on the artificial diet for the Danainae butterfly is relatively rare in Taiwan. This could be improved if, for instance, based on the results of our experiment, the linseed oil was added into the artificial diet and the composition was adjusted to create a more balanced nutrients, improved formulation to raise the survival rate and shorten the developmental period of giant danaine butterfly reared with the artificial diet. We expect a successful transition into the commercial production of the diet in the future, so that the production of this butterfly species would be freed from the limitations of seasonal variation and host plant supply. This species can be used as the material for school children to rear and observe its life history. It can also be used as teaching material for other relational courses. Furthermore, mass production and reproduction of the giant danaine butterfly can serve as other research and teaching purposes. By utilizing its good ecological and exhibition effect, this butterfly species can be used in leisure vacation farms and butterfly gardens as well as other such attractions.

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# Appendix

App. Table 1 Composition of the semi-synthetic artificial diet for *Idea leuconoe clara*

| Ingredients | Quantity | Ingredients | Quantity |
|---|---|---|---|
| Common bean | 50.00 g | Ascorbic acid | 0.90 g |
| Dried leaf powder | 26.00 g | Sorbic acid | 0.30 g |
| Wheat germ | 14.00 g | Vitamin | 0.80 g |
| Yeast | 16.00 g | Wesson salt mix | 2.00 g |
| Casein | 15.00 g | Choline chloride | 0.80 g |
| Gelcarin | 40.00 g | Methyl paraben | 0.50 g |
| Sucrose | 5.00 g | Tetracycline | 0.01 g |
| Glucose | 12.50 g | Formaldehyde | 1.00 ml |
| | | Distilled water | 825.00 ml |

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