Larval Development and Survival of Mimetic and Non-Mimetic Female *Papilio polytes* Linnaeus, 1758 (Lepidoptera: Papilionidae) Reared in Captivity in Taiwan[1]

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Abstract: Females *Papilio polytes* Linnaeus, 1758 (Papilionidae), the common Mormon butterfly, exhibit two distinct forms: one resembles conspecific males; the other resembles an unpalatable model, *Pachliopta aristolochia* (Fabricius, 1775) (Papilionidae), to which *P. polytes* females are often assumed to be Batesian mimics. Although females *P. polytes* carrying the gene for mimicry have a greater chance of avoiding predation, the mimetic gene is accompanied by trade-offs such as the number of eggs laid, hatching rate, larval survival rate, and adult lifespan. Based on 400 *P. polytes* larvae reared under captivity on *Citrus limon* (Linnaeus) Osbeck (Rutaceae), the mimetic form develops faster and some of its instars are longer than their non-mimetic counterparts. Also, the number of offspring (larvae and pupae) from mimetic females is lower (although not statistically significant) than the number from non-mimetic females. Hence, the retention of the mimetic gene in wild populations is consistent with the hypothesis that the benefit of adult mimicry outweighs the physiological maladies that could be manifested in the larvae and/or pupae of mimetic *P. polytes*.

Key Words: Larval development, adult survival, mimicry, mimetic and non-mimetic female *Papilio polytes*, *Pachliopta aristolochia*, trade-offs, Papilionidae, Lepidoptera, captive rearing, Taiwan, genetics

Introduction

When rearing *Papilio polytes* Linnaeus, 1758 (Papilionidae), also known as Common Mormon butterfly, I observed two wing phenotypic patterns in females: one with large red and white patches on the hindwings presumably mimicking another species of butterfly; the other without such patches (Figure 1). The mimetic *P. polytes* renders those females similar to the Common Rose butterfly, *Pachliopta aristolochiae* (Fabricius, 1775)*, a species reported as unpalatable to birds (Euw et al. 1968). The flight patterns of the mimetic and non-mimetic Common Mormon female butterflies are also different, with the mimetic *P. polytes* butterflies flying like to their model (Kitamura and Imafuku 2015). The pre-adult stages of the mimetic female offspring have also been shown to have higher mortality than the offspring of non-mimetic females (Katoh et al. 2020).

Does the offspring from mimetic females in Taiwan have a lower survival rate during the larva and pupal stages? Using the Common Mormon butterfly, *P. polytes*, I designed an experiment to answer this question and explored how the mimetic genes affects the species, specifically in the larval stage.
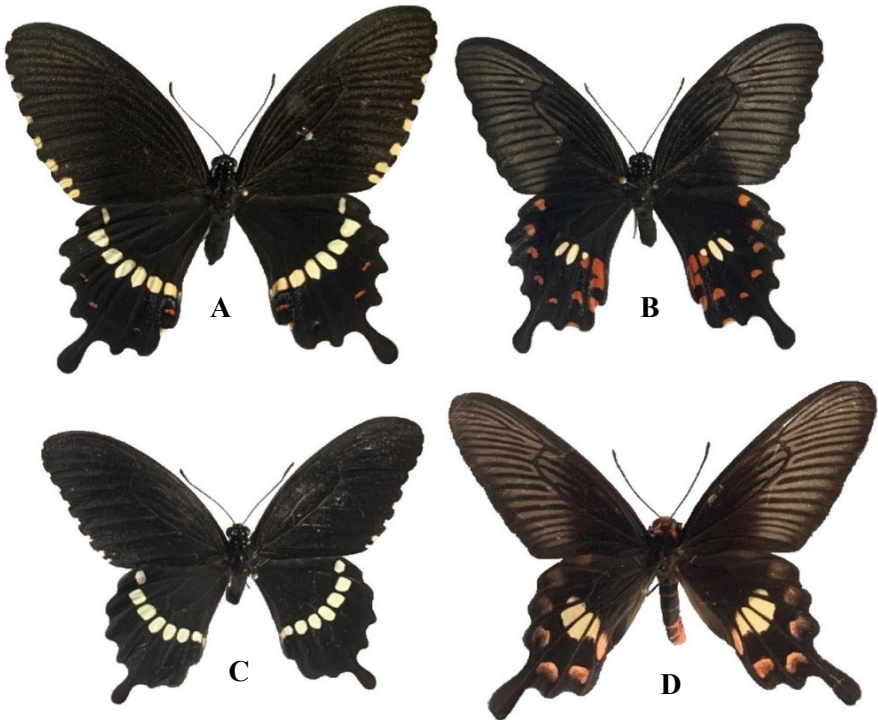

Figure 1. Dorsal views of adult *Papilio polytes* (A-C, reared in the laboratory) and *Pachliopta aristolochiae* (D, collected in the wild) showing wing coloration pattern. A. F_2 non-mimetic female. Wingspan is 95 mm. B. F_2 Mimetic female. Wingspan is 80 mm. C. F_2 Male. Wingspan is 77 mm. According to Islam et al. (2017), the average wingspan for females and males *Papilio polytes* reared in laboratory conditions are 95.2 ± 6.19 mm and 87.4 ± 6.15 mm, respectively. D. *Pachliopta aristolochiae* female, the mimicry model for *Papilio polytes*. Wingspan is 86 mm. The wingspan for adult Common Rose, *Pachliopta aristolochiae*, varies from 80 - 110 mm (Atluri et al. 2001). Photographs by the author.

The Common Mormon butterfly appears to be highly palatable to aerial hawking birds, such as *Hypsipetes amaurotis* (Temminck, 1830) (Pycnonotidae), known as the Brown-eared bulbul (Uésugi 1996). *Papilio polytes* females of the "typical", or non-mimetic, form are attacked in midair more frequently than males (Ohsaki 1995). Data collected in Borneo (Indonesia)

and Malaysia showed that females with a non-mimetic wing pattern have a much higher rate of beak marks left by bird attacks than female with mimetic wing pattern (Ohsaki 1995). Also, Ohsaki (1995) concluded that bird predators attack females more frequently because their larger abdomens make them less agile and, hence, easier to capture than their conspecific males. Birds are visually orienting predators and usually select their prey based on colors and patterns (Jeffords et al. 1979). Hence, having a wing pattern that reduces attacks from birds would give significant selective advantage to the organisms possessing those traits.

In research involving prey selection of birds in India, the Common Mormon butterfly is described as a species in Mullerian mimicry with the Common Rose, *Pachliopta aristolochiae* which has aristolochic acids[3] in the body tissue. However, other studies suggest that the Common Mormon is palatable to predators and that the mimetic female is recognized to be a Batesian mimic of the Common Rose (Ghosh and Saha 2016, Kunte 2007, Uésugi 1996). In both Mullerian and Batesian mimicry systems, the evolution of aposematism presumably represents a selective advantage to the prey because it presents chromatic attributes considered by the predator to signal distasteful or dangerous qualities. This warning pattern can be beneficial both to the potential prey organisms, by increasing their survival rate, and to the potential predators, by avoiding the consumption of unpalatable prey (Malcolm 1990). I analyzed the survival rate of Common Mormon butterfly, *Papilio polytes*, based on possible Batesian mimicry with Common Rose as the model species.

The Common Mormon butterfly exhibits two female wing color forms: one that is similar to the male (Figures 1A and 1C), and the other that apparently is a Batesian mimic (Figure 1B) of the unpalatable *Pachliopta aristolochia*, the Common Rose swallowtail (Figure 1D). In this case, Common Mormon, *Papilio polytes*, is suggested to have evolved faster towards the Common Rose model, *Pachliopta aristolochia*, instead of away from it. Presumably this evolution towards the model is an advantage in Batesian mimicry (Nur 1970). A study conducted in the Ryukyu Islands (Japan) suggested that the abundance of mimics is limited by the abundance of the models and mainly based on negative frequency-dependent selection from predation pressure (Tsurui-Sato et al. 2019). With abundant model species in the area, the predators would recognize the wing color pattern and the behavior of unpalatable species, and reduce the attacks towards the female Common Mormon, *Papilio polytes*, with mimetic wing patterns.

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[3] Aristolochic acids, "known human carcinogens" (Anonymous 2019, 2021), are present in plants of the family Aristolochiaceae. Several host plants of the Common Rose butterfly, *Pachliopta aristolochiae* are members of the genus *Aristolochia*. The Common Rose butterfly has aristolochic acids (Euw et al. 1968).

Increased survival of *P. polytes* mimetic females should provide increased opportunities to reproduce and pass along the mimicry trait. However, previous research in Japan indicates that this advantage in predator avoidance is accompanied by physiological trade-offs. That is, the mimicry gene of *Papilio polytes*, is related to decreases in the number of eggs laid, hatching rate, larval survival rate, or adult lifespan among the Common Mormon populations in Japan (Ohsaki 2005, Katoh 2020, Komata et al. 2020).

Mimicry is likely to evolve in situations where the balance of costs and benefits is favorable. This study focuses on the Common Mormon, *Papilio polytes*, and it examines whether there are similar physiological trade-offs among the populations from one population in Taiwan. Could this balance tilt in favor of the Common Mormon population to continue carrying the non-mimetic genes in the wild (Ohsaki 2005)? If that were the case, does the larval offspring of Batesian mimics have a higher mortality due to physiological trade-offs? An alternative outcome would be that the offspring from mimicry and non-mimetic females having similar survival rates before adulthood without detectable trade-offs resulting from the mimetic gene to the individuals carrying it.

Methods

Species studied. The Common Mormon, *Papilio polytes*, is a species of swallowtail butterfly that occurs in India, Southeast Asia, China, Korea, and Japan (Omura et al. 2006). Studies on the biology of the species have been conducted in Japan (Komata et al. 2020), India (Atluri et al. 2002), Malaysia (Jaafar et al. 2013), Taiwan (Chen and Ouyang 2007), and Pakistan (Munir 2004). In Taiwan, where this study was conducted, this species can be found from March to October with peak adult (butterfly) populations typically from June to August. The species is multivoltine with up to five generations annually (Chen and Ouyang 2007).

Life history of Papilio polytes. The eggs usually hatch in three days and the larvae go through five to six instars before the prepupal stage (Figure 2). The pupa requires an average of ten days to emerge, however, low temperatures, *P. polytes* may diapause through winter in the pupal stage (Chen and Ouyang 2007). A study of a congeneric, *Papilio demoleus* Linnaeus, 1758, noted that some species of butterfly can enter diapause when the temperature drops below their optimal range, and that the diapause can last up to six times longer than the pupal period of warmer months (Islam et al. 2019). The average developmental time for a single generation of the Common Mormon is 28-30 days (Atluri et al. 2002, Jaafar et al. 2013) in India as well as Malaysia, and 33-36 days in Taiwan (Chen and Ouyang 2007). Figure 2 shows the different life stages of the Common Mormon, including egg, third instar larvae, fifth instar larvae, prepupa, and pupa. The chromatic variation of *Papilio polytes* pupae mostly depends on

the environmental conditions experienced by a pupating larva; brown pupae are usually found on rough and darker color surfaces, green pupae are usually found on smooth, lighter color surfaces (Smith 1978).
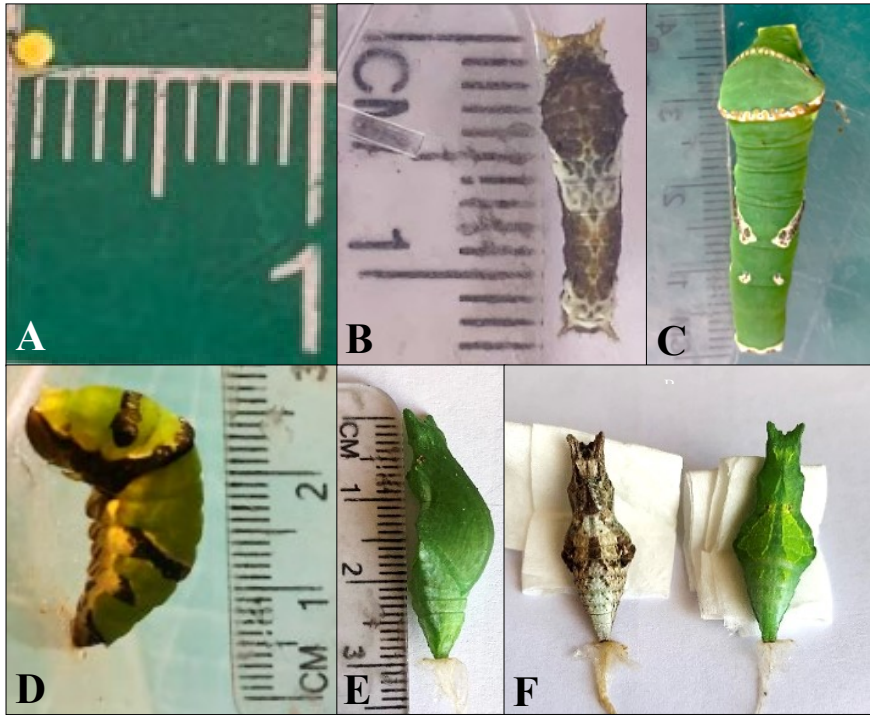

Figure 2. Common Mormon butterfly, *Papilio polytes*, life history stages. A. Egg. B. Third instar larva. C. Fifth instar larva. D. Prepupa. E. Pupa. F. Color variation of pupae. The first three larval instars are black, the fourth instar is grayish, and the fifth instar is green. All instars have white patterns dorsally.

This female-limited Batesian mimicry in the Common Mormon butterfly is genetically linked to a single autosomal locus H; the mimetic trait (H) is dominant to the non-mimetic trait (h) (Clarke and Sheppard 1972). Female *P. polytes* are polymorphic: HH or Hh females have mimetic wing patterns (Figure 1B); hh females have non-mimetic wing patterns. However, the locus H does not affect the male wing color phenotype; HH, Hh, and hh males all have monomorphic non-mimetic wing patterns (Iijima et al. 2019).

Captive Rearing. Herein, I describe the typical laboratory protocols followed for the experiments herein described. In Taiwan, where I do most of my research on butterflies, the eggs, larvae, and host plants are usually field collected or purchased online. During the larvae stage, caterpillars are kept in containers with

sufficient airflow and indirect sunlight, with host plants placed in bottled tap water. The rearing containers are cleaned with 75% ethanol daily to minimize the likelihood of diseases. The host plant leaves are replaced daily to ensure fresh food sources for the developing larvae. When reaching the final larval instar, it is important to provide enough space for the larvae to move around the container and find a suitable spot for pupation. Pupae are carefully moved to a butterfly cage where they emerge. For different species of *Papilio*, emergence occurs usually around ten days following pupation, but this time depends on environmental factors, such as temperature. After emergence, the newly emerged butterfly takes 30% honey water as the main food source. A dish containing honey water was placed inside the butterfly cage to keep food source accessible to the butterflies.

Reproduction through hand pairing. To perpetuate the species through hand pairing, it is important to ensure that the adults are sexually mature. For *Papilio*, the male butterfly is sexually mature after three days; the female is sexually mature right after emergence. After successful copulation, the female is placed on her larval host plants for oviposition and egg collection. Males die soon after copulation. *Papilio* eggs can be hand collected from the host plant and placed in another container for hatching. *Papilio* larvae hatch three days after oviposition and were moved onto the host plants for rearing. Appendix 1 shows images of my work rearing butterflies.

Experimental protocols for captive rearings. Three *Papilio polytes* larvae were collected in Kaohsiung City (southern Taiwan) on leaves of *Citrus limon* (Linnaeus) Osbeck and taken indoors for observation. Many members of the genus *Papilio* utilize plants in the family Rutaceae as larval hosts (Shobana et al. 2010). All the *P. polytes* larvae were reared on *C. limon*.

After reaching adulthood, the three individuals (Figure 3) were hand paired (Clarke and Sheppard 1956). Due to the uncertainty of the parental genotypes, the male genotype could be HH, Hh, or hh, and the mimetic female genotype could be HH or Hh, the resulting eggs were grouped based on the parental phenotypic of the mimetic female (HH or Hh) and the non-mimetic female (hh). The same male butterfly was used for breeding to limit the potential differences in male parental genotypic differences. Although the order of copulations was not recorded in the experiment, each copulation took place a day apart.

In this experiment, group 1 represented the offspring from the mimetic *Papilio polytes* female, and group 2 represented the offspring from the *P. polytes* non-mimetic female. In the first replicate, mated females were placed in butterfly cages containing freshly cut *C. limon* branches containing leaves and placed in small jars containing water (Figure 4). The females were isolated to obtain eggs and the first 100 larvae hatched from each group were used for collecting survivorship data. Additional eggs were collected to record the number of eggs laid and the hatching rate. The females were later kept in the butterfly cages to

record the differences in the adult lifespan. Each female was fed with honey water (Glass and Pan 1983) and their survivorship was checked daily.
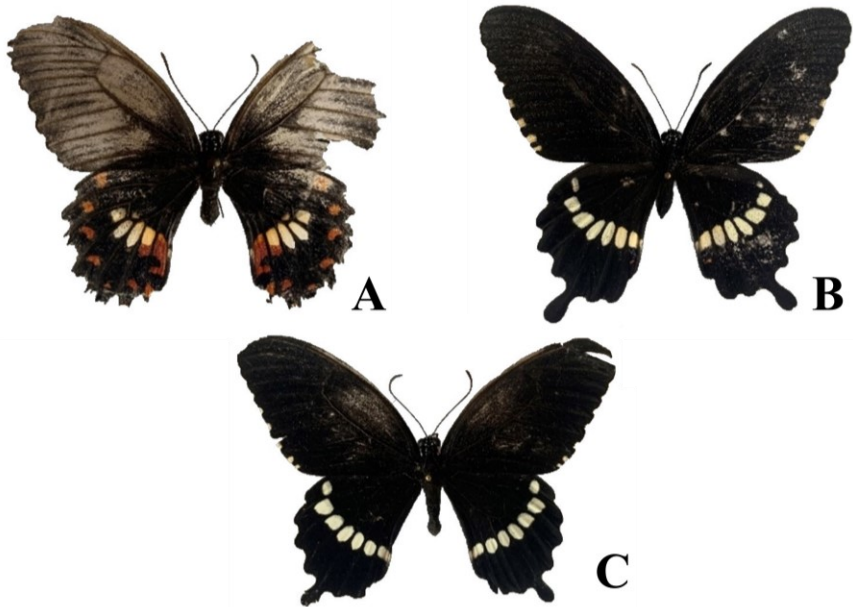

Figure 3. The three original (parental) *Papilio polytes* butterflies used in the study. A. Parental mimetic female. B. Parental non-mimetic female. C. Parental male.

In the second replicate, a pair of adults were selected from the offspring of the parentals from each group and set aside for reproduction. Although the genotype for males remained uncertain, a female with mimetic wing pattern (HH or Hh) in group 1 was selected and a female with non-mimetic wing pattern (hh) from group 2 were selected. Two male adults were selected from each group respectively and paired with the female form the same group. The observations took place from September 14 to December 14, 2021. The first replicate was done from September 14 to November 2; the second replicate from October 26 to December 14.

Data Collection. In each replicate, one female from each group were used for eggs collection (total of four females used in this experiment) and the collected eggs were separated into different glass containers for hatching (Figure 4). Usually, eggs hatch in three days, and those that did not hatch after seven days were considered inviable. Hatched larvae were kept inside rearing containers and were used to obtain data for larval survival rates. Host plants species can influence the growth and reproduction of butterflies (Shobana et al. 2010), therefore, the food source offered was limited to *Citrus limon*. In

addition, due to the limitation of food sources, only a total of 400 larvae, 200 larvae each generation, were part of the experiment.
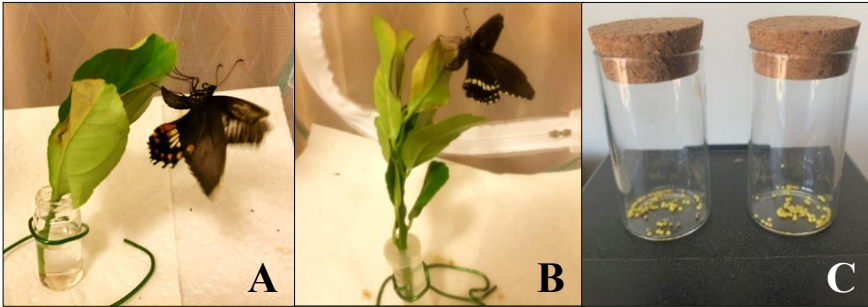

Figure 4. *Papilio polytes* females laying eggs inside a butterfly cage and eggs collected. A. Mimetic female. B. Non-mimetic female. C. Eggs from mimetic and non-mimetic female separately.

Once hatched, an average of 10 larvae that hatched the same day were placed in one Petri dish and supplied with fresh *C. limon* leaves daily. Upon reaching the third instar, the larvae were moved into a 1-liter container. Mortality was checked daily, and the larval body length was recorded daily by selecting ten randomly chosen individuals from each group in each instar.

The pupal stage for *Papilio polytes* usually lasts 10 days, however, *P. polytes* tends to diapause as a pupa when the environment is not suitable (e.g., during the winter). In the first replicate, pupae that did not hatch in 30 days or became visibly putrid were considered dead. These specimens were included in the survivorship calculations. Most second replicate pupae took longer than 10 days to hatch due to low environmental temperatures, thus the survival rate was calculated using 49 days in the first replicate and 59 days in the second replicate of the larvae and pupa development. Emerged adults from the parental generation and F_1 were used for pairing after sexually mature. For the Common Mormon, *P. polytes*, males were sexually mature three days after they emerged from the pupa; female butterflies were mature right after emergence (Fryer 1914).

Room temperature and humidity were recorded daily (Xiaomi Mijia Bluetooth thermometer and hygrometer, China) using a thermometer and hygrometer placed with the butterflies. The environmental temperature and humidity were collected through the Hsinchu weather monitoring station, retrieved from the Central Weather Bureau (CWB) Observation Data Inquire. The number of larvae was recorded daily and used to construct a survivorship curve to compare the survival rate between two groups.

Statistical Analyses. Differences in number of eggs laid, number of eggs hatched, adult longevity, and cumulative number of deaths were assessed with

the Welch test. The Welch test is a modification of Student's t-test that allows testing statistical differences between groups with unequal variances with small sample size (n ≤ 5). In a Welch test, p values ≤ 0.05 are considered statistically significant (De Winter 2013). Differences in survival patterns between larvae from the different parental phenotypes were assessed using the log-rank test (Gotthard et al. 2000). The data of each replicate were analyzed separately using the log-rank test. The log-rank test allows comparison of the survival of groups, and it is most likely to detect a difference when the risk of an event is consistently greater for one group than another (Bland and Altman 2004). Each replicate consisted of 200 individuals, for a total of 400 individuals in two replicates. In these analyses, the time (in days) between egg hatch and adult emergence is the quantity of interest; the risk event is death. The degrees of freedom are the number of groups minus one, which, in this case, is one.

Results and Discussion

Temperature and humidity. The average temperature ranged from 19.8°C to 35.7°C and the humidity ranged from 37% to 98% (Table 1). The recorded temperature and humidity for the first replicate are relatively higher when compared to the second replicate. There seems to be a trend of decreasing temperature and humidity from September to December.

Table 1. Average temperature and humidity during September to December 2021. Room temperature and humidity were measured using a thermometer and hygrometer placed with the butterflies; environmental data were retrieved from CWB Observation Data Inquire System.

	Air temperature (°C)			Humidity (%)		
	Room		Environmental	Room		Environmental
	Maximum	Minimum	Mean	Maximum	Minimum	Mean
September	31.3	24.7	29.3	98	58	73
October	32.1	23.1	26.0	87	44	71
November	35.7	19.8	21.1	86	37	76
December	23.9	20.2	18.6	76	51	66

The low temperature in November and December possibly affected the survivorship and the developmental time for the butterfly in the second replicate. Most swallowtail butterflies (Papilionidae), including Common Mormon, prefer warmer temperatures and less precipitation. Feeny et al. (1985) showed that larval survivorship could be twice as high under higher monthly mean temperature of 20.6°C and lower monthly total precipitation of 5.38 cm for the Black Swallowtail butterfly in central New York.

Reproduction and adult genotype. The inferred genotype of females and the potential genotype for male butterflies are shown on Figure 5.

In group 1, based on the female wing patterns in the F_1, the parental mimetic female has the genotype of Hh and the male in parental either has the genotype of Hh or hh, allowing the present of the non-mimetic wing pattern female in F_1. After crossing a female with mimetic wing pattern and a male from F_1, the results of F_2 have both mimetic and non-mimetic wing pattern female. Based on the result, the F_1 mimetic female also carries a genotype of Hh and the F_1 male has to carry either Hh or hh genotype. In F_2, the male genotype remains uncertain (HH, Hh, or hh) and the female genotype with mimetic wing pattern could be HH or Hh, but the genotypic differences in F_2 will not affect the data collection result for larvae mortality rate in the two replicates.

In group 2, the non-mimetic wing pattern female for the three generation all carries hh genotype. There is no female with mimetic phenotype present in all three generations in group 2, however, according to the previous analysis in group 1 the male in parental carries either Hh or hh genotype. It is possible that all offspring carrying mimetic genotype are male or did not make it to adult stage. Therefore, the genotype of male in group 2 remains uncertain.
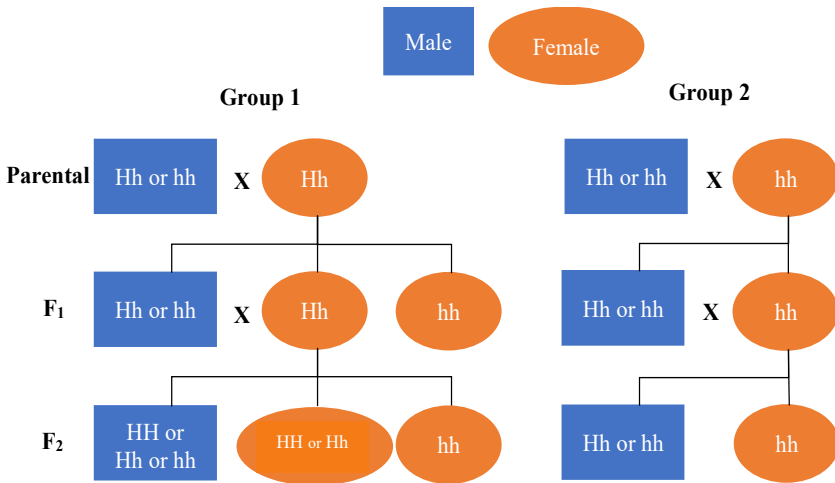

Figure 5. Genotype for both groups in the three generations. Group 1 females are mimetic, group 2 females are non-mimetic.

Number of eggs laid, hatching rate, and adult lifespan (Table 2). A total of 1,018 eggs were obtained and calculated for the number of eggs laid in the two replicates. Among those eggs, a total of 819 larvae hatched.

Table 2. Number of eggs laid, number of eggs hatched, and adult longevity for both groups in two replicates. To reduce the uncertainty of the experiment the same male was used in the first replicate. Group 1 females are mimetic, group 2 females are non-mimetic. None of the Welch tests were statistically significant.

	Number of Eggs Laid		Number (percentage) of Hatched Larvae		Adult Longevity (days) (Four females and three males used)			
					Female		Male	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
First Replicate	287	341	190 (66%)	295 (87%)	27	33	11	
Second Replicate	208	182	172 (83%)	162 (89%)	26	30	9	9
Total Eggs	1,018 oviposited		819 hatched larvae					

The actual number of eggs laid was higher for the mimetic female in the first replicate but higher for the non-mimetic female in the second replicate. The number of eggs laid in the second replicate was lower than the number of eggs laid in the first replicate for both groups. However, these differences are not statistically significant (p = 0.90). Perhaps, this was caused by competition for limited food sources during the egg-laying females' larva stages. When less food s consumed or when there is a low efficiency transforming food to body mass, the body size in the adult stage and the number of eggs that a female can lay will decrease (Shobana et al. 2010). The parental *Papilio polytes* females were collected in the wild. The larvae from those females (F_1) were reared in groups of five conspecifics which could have experienced reduced competition as compared to the females whose larvae (F_2) were reared with ten conspecific larvae in the same container. Another possible explanatory factor could be the temperature differences. The first replicate was done from September to October, and the second replicate was done from October to December. The average temperature is lower in Taiwan from October to December, leading to decreased activity for adult butterflies and some of the Common Mormon in the wild will stay in the pupal stage and emerge in the following spring (Chen and Outang 2007).

The percentage of eggs hatched was higher for the eggs laid by a non-mimetic female in both replicates, and the hatch rate is higher in the second replicate for both groups when comparing the two replicates. These differences are not statistically significant (p = 0.607).

The adult longevity was shorter for the mimetic females (27 days and 26 days) and longer for the non-mimetic females (33 days and 30 days). These differences are not statistically significant (p = 0.194). Although this result is not

statistically significant, the longer adult lifespan allows more opportunities for non-mimetic females (Group 2) to lay more eggs in captivity in the absence of predators. In a recent study in Japan, Komata et al. (2020) found that adult longevity of Common Mormon, *Papilio polytes*, is highest in hh genotype females and lowest in HH genotype females when there are no predators. The results herein presented for males are compatible with those of Komata et al. (2000) who suggested that male butterflies do not have significant differences in longevity among individuals carrying different genotypes.

Male butterflies usually have significantly shorter life spans compared to females (Gotthard et al. 2000). In my experiment, males lived for 11 days and 9 days, respectively. The Batesian mimicry genotype does not change the phenotype for male butterflies and all the males show non-mimetic wing patterns. The non-mimetic wing pattern can potentially increase the predation pressure in the wild. The actual number recorded for eggs laid, number of eggs hatched, and female adult lifespan are consistent with the hypothesis that a possible trade-off for Batesian mimicry although the differences were too small to be statistically significant.

Larval Development. Larval development was measured using only individuals that successfully reached the adulthood offspring from females with and without the mimetic gene (Table 3, Figure 6). No individuals entered diapause in the experiment.

Table 3. Observed larval developmental time (in days) for both replicates. Group 1 females are mimetic, group 2 females are non-mimetic.

Developmental time (days)						
Life stage	Group 1		Group 2		All Individuals Combined	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
First replicate						
Larva	27.2 ± 2.2	23~30	25.5 ±3.8	21~32	21.6 ± 3.3	21~32
Pupa	12.0 ± 1.0	10~13	11.5 ± 1.3	9~14	11.6 ± 1.2	9~14
Egg to Adult	38.9 ± 3.0	33~43	37.0 ± 5.0	30~46	37.6 ± 4.4	30~46
Second replicate						
Larva	29.7 ± 4.1	25~40	27.0 ±3.9	20~39	28.2 ± 4.1	20~40
Pupa	17.9 ± 0.7	17~19	17.7 ± 1.2	16~20	17.8 ± 1.1	16~20
Egg to Adult	47.5 ± 4.4	42~58	44.1 ± 4.6	36~47	45.2 ± 4.6	36~58

When exposed to the same resources and rearing conditions, offspring lacking mimetic genes have a faster growth rate, either through a higher rate of utilizing nutrient intake or through consuming food at a faster rate.
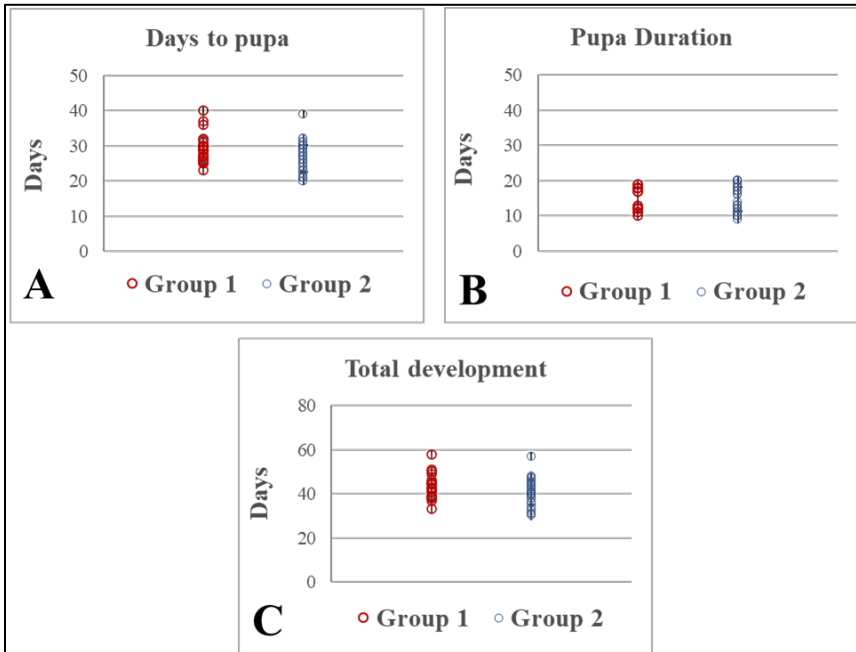

Figure 6.   Developmental time for the larvae, pupa, and total time for individuals successfully metamorphosized in both replicates combined. A. Days to pupa. (= egg to larva). There is a significant difference in the developmental time between the mimetic (group 1) vs the non-mimetic group (t-test, p < 0.05). B. Pupa duration. C. Total developmental time. Group 1 females are mimetic; Group 2 females are non-mimetic. There is no significant difference in the pupal developmental times and in the total developmental time (C) between the mimetic (group 1) vs the non-mimetic groups.

The developmental times for both groups are longer than the time reported by Chen and Ouyang (2007) in Taiwan (33.9 ± 0.4 days) and Jaafar et al. (2013) in Malaysia (28.3 ± 1.9 days). Temperature, host plant species, competition among individuals in the same container, or the researcher's manipulation of the specimens could have caused these differences. In the experiments conducted in Taiwan and Malaysia the temperature ranged from 25°C to 32°C, which had a higher minimum temperature than the temperature recorded here (23.1°C to 32.1°C). Geographical variation can also affect the time for larval and pupal development, causing longer development time or diapause for pupa during winter

(Yoshio and Ishii 1998). Day length could also influence the development of *P. polytes*. A previous study on *Papilio xuthus* Linnaeus suggests that a shorter day length would lead to longer larval developmental time (Komata and Sota 2017).

The developmental time was significantly longer during the pupal stage in the second replicate, and since the experiment was conducted in the same location, temperature differences likely are the main factor causing the difference. The average temperature readings recorded ranged from 23.1°C to 32.1°C and the humidity ranged from 44% to 98% in the first replicate. The temperature ranged from 19.8°C to 35.7°C and the humidity range from 37% to 86% in the second replicate. Although the experiment was conducted indoors, the temperature and humidity fluctuated with the local weather and possibly causing the longer pupa developmental time. A study of temperature effect on larval development and survival in Taiwan reported a low temperature threshold for the development of egg, 1st, 2nd, 3rd, 4th, 5th instar larvae, and pupae were estimated to be 10.33, 13.42, 12.74, 12.65, 10.56, 9.58, and 12.42°C (Chen et al. 2000). Although the temperature in this research did not reach the low threshold, the higher number of deaths and the longer pupal developmental in the second replicate could have been caused by these slightly lower temperatures.

Host plants' water, protein, nitrogen, carbohydrate, and lipid contents can influence the larvae development, growth rate, and adult longevity. Water and nitrogen content is the main factor that influences growth rate, and the high protein content usually leads to more successful development in the Common Mormon larvae, *P. polytes* (Shobana et al. 2020). Chen and Outang (2007), reared *P. polytes* larvae on *Citrus sunki* hort. ex Tanaka (Rutaceae), Jaafar et al. (2013) reared larvae using *Murraya koenigii* (Linnaeus) Jack (Rutaceae), and I reared the larvae on *Citrus limon*. The nutrient contain for the food source in this experiment is unknown, but the difference in host plant could be a factor causing a longer developmental period. Although Common Mormon larvae only feed on plants in the family Rutaceae, adult females may have preferences on the plant species they lay eggs on. In the wild, female butterflies usually have their host plant prefereences for oviposition, and they detect specific chemicals through their forelegs when contacting the leaf surface (Ozaki et al. 2011).

Climate, weather, and diets are regional differences difficult to control in rearing butterfly larvae, but other anthropic factors could impact the experimental results unintentionally. This experiment eliminates the predation pressure by keeping the larvae indoor, but alternatively with more frequent contact with humans. This factor could make the larvae be more alert of potential predation when human is present, leading to slower development for larvae reared indoors. Gotthard (2000) studied predation pressure and growth rate on speckled wood butterfly, *Pararge aegeria* (Linnaeus, 1758) (Nymphalidae), larvae and found that some larvae grow faster by increasing the

time spend on feeding when there is a high fitness to the habitat and trade-off with a higher risk of death from predation. The fast-growing larvae that survived despite predation risks producing one more generation.

Some individuals in group 2 have faster larval development than others when provided with the same resources. The intraspecific competition might cause uneven food distribution among individuals in the same container, causing differences in developmental speed. It would be interesting to conduct experiments on the resource utilization between the offspring from mimetic females and non-mimetic females to test whether one of the trade-offs for Batesian mimicry is being less competitive.

Larval body length. Butterfly larvae usually grow geometrically in linear dimensions with every molt (Gotthard 2000). The larval length ranges from 2 mm to 52 mm and the two groups have similar growth patterns (Table 4). The mimetic larvae are statistically longer in the second and fifth instars (t-test, $p <$ 0.05) than the non-mimetic ones. There were no statistical differences in length between the mimetic (group 1) and non-mimetic larvae (group 2) for the other instars. Fifth instar larvae can consume 76.9% of the total food consumed during the larval period, leading to more growth in body length as compared to the other instars (Chen and Ouyang 2007).

Table 4. Observed larvae length by instar. Group 1 females are mimetic, group 2 females are non-mimetic.

Larval Length (mm)				
Life stage	Group 1		Group2	
	Mean ± SD	Range	Mean ± SD	Range
1st instar	4.0± 1.1	3~6	4.2± 1.1	3~6
2nd instar	9.9± 1.4	8~13	8.7± 1.5	7~12
3rd instar	18.2± 1.4	15~22	17.7± 4.0	12~27
4th instar	28.4± 2.7	25~35	27.0± 2.8	22~33
5th instar	46.3± 4.1	40~52	42.8± 6.0	30~51
Pupa	28.5± 1.7	25~31	28.0± 2.4	22~31

When compared to larvae with the mimetic gene (group 1), larvae carrying non-mimetic genes (group 2) appear to take shorter time to develop (Table 3) and have a slightly longer body in the second the fifth instar (Table 4). The results for the Common Mormon larval body length in this research resemble the results from Komata et al. (2020); one of the trade-offs for the mimetic phenotype is reduced larval performance in their offspring. Larvae with a mimetic gene have lower growth efficiency, longer developmental periods, and lower growth efficiency.

Survivorship Curves (Figure 7) In the first replicate, the overall developmental period was 49 days; in the second replicate, the overall developmental period was 59 days. However, although there were no significant differences in the survival curves between the mimetic and the non-mimetic groups, the cumulative number of dead caterpillars and pupae appear different between the two groups.
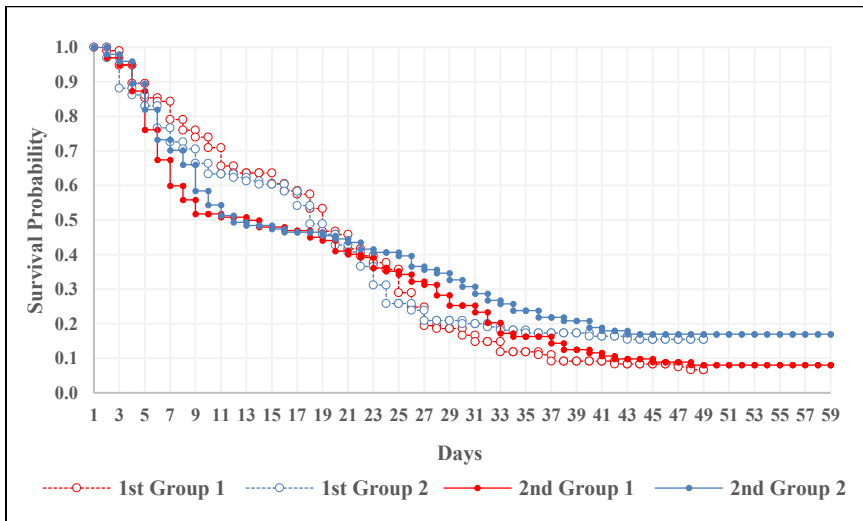

Figure 7. Common Mormon survivorship curve for both replicates (1st, 2nd) separated by groups (group 1 females are mimetic, group 2 females are non-mimetic). In both replicates, there are no statistical differences between the survivorship curves between the mimetic and the non-mimetic groups (log-rank test, $\chi 2$, $p > 0.05$).

In both the mimetic and non-mimetic groups, approximately half of the larvae died in the first 20 days of the experiment, suggesting that, during the younger instars, the larvae are more vulnerable (Figure 8). Field research of butterfly larvae in Sweden reported that 54% of *Pararge aegeria* larvae in the field disappeared during the first six days after hatching, showing that early-stage larvae are more fragile (Gotthard 2000). In my experiments, the larvae are protected from the outdoors and there are no predators, the percentage of dead larvae counted in the first six days in this experiment is much lower than the percentage of larvae that disappeared in Gotthard's (2000) field experiment. These results further support the hypothesis that a major cause of butterfly larvae mortality is predation. After day 21, the number of deaths in group 1 in both replicates increased faster than that of group 2. This result is consistent with what Katoh et al. (2020) report, namely that individuals carrying mimetic genes have a higher mortality rate at the final instar and therefore have a lower

overall survival rate. The cumulative number of dead accumulate slower after day 33 when compared to earlier stages, showing that the pupal stage and the adults have lower risks of failure during development.
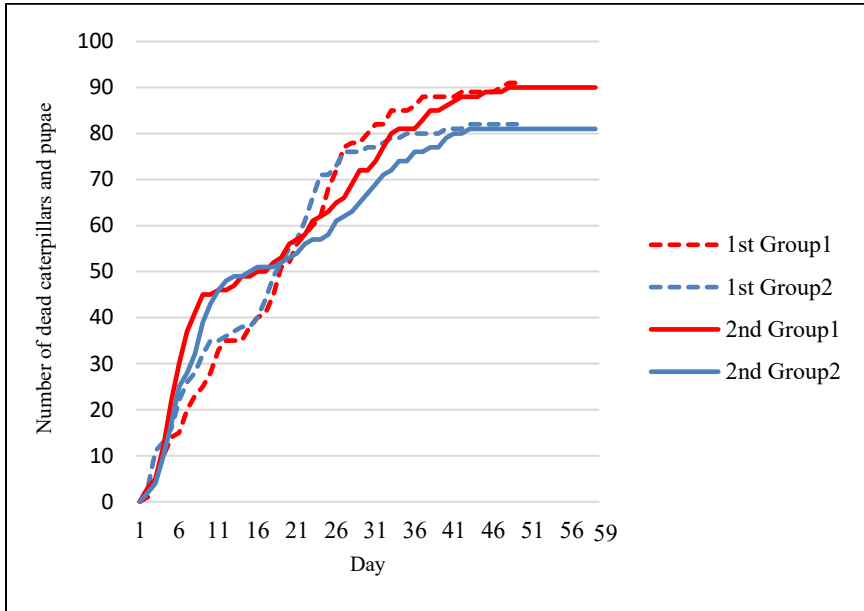

Figure 8. Cumulative number of dead caterpillars and pupae in two replicates by days. Group 1 (in red) females are mimetic, group 2 (in blue) females are non-mimetic. Welch tests were significant between the two groups, p < 0.05 in both replicates.

The cumulative number of dead larvae and pupae from the mimetic females (group 1) was higher than the non-mimetic females (Welch test, p = 0.006). In both replicates, the total number of adults that emerged from group 2 was larger than the adults that emerged from group 1. In the first replicate, there nine offspring of the mimetic female successfully metamorphosized, and three of the female offspring showed mimetic wing patterns. A total of 18 offspring of the non-mimetic female successfully metamorphosized and none of them showed a mimetic wing pattern. The total survival rate in group 2 (18%) was twice higher than the survival rate in group 1 (9%, Tables 5 and 6).

In the second replicate, ten offspring from the mimetic female successfully metamorphosized, but only two of the females showed mimetic wing patterns. In contrast, 19 offspring of the non-mimetic female successfully metamorphosized and none of them showed a mimetic wing pattern.

Table 5. Number of individuals reared and emerged as adults in the experiment with Common Mormon, *Papilio polytes*. The number of individuals successfully developed to adults is higher in group 2. Group 1 females are mimetic, group 2 females are non-mimetic.

	Group 1	Group 2
First replicate (n =200)		
Male adult	2	9
Mimetic female	3	0
Non-mimetic female	4	9
Total number of emerged	9	18
Second replicate (n =200)		
Male adult	5	12
Mimetic female	2	0
Non-mimetic female	3	7
Total number of emerged	10	19
Total (n = 400)		
Male adult	7	21
Mimetic female	5	0
Non-mimetic female	7	16
Total number of emerged	19	37

Komata et al. (2020) suggested that the larval survival rate of hh females crossed with hh and Hh males is significantly higher than those of larvae of other females. The larvae survival rate with Hh female crossed with Hh or hh male has a lower survival rate, and the larval survival rate from HH female crossed with any other male genotypes is the lowest. In this experiment, larvae in group 2 (non-mimetic) are the offspring from hh female crossed with Hh or hh male (male genotype is uncertain), which is the group with the highest survival rate in Komata et al. (2020). The mortality rate during the larval period is 87% in group 1 (mimetic) and 82% in group 2. In the second replicate, a female (Hh or HH) with a mimetic wing pattern from group 1 was crossed with a male (Hh or HH) from group 1, and a female (hh) with a non-mimetic wing pattern from group 2 was crossed with a male (Hh or hh) from group 2. Based on the results from Komata et al. (2020), it is expected that the survival rate for the third-generation in group 2 will still be higher than the survival rate in group 1. The mortality rate in the second replicate during the larval period was 80% in group 1 (mimetic) and 76% in group 2 (non-mimetic). The total mortality rate for the experiment was 90% in group 1 and 81% in group 2, more individuals from a non-mimetic female successfully reached adult. No larva overwintered as pupa.

Table 6. Mortality rate in both groups separately in two replicates. The mortality rate is higher in group 1 in all stages. Group 1 females are mimetic, group 2 females are non-mimetic.

	Group 1	Group 2
First replicate		
Larvae mortality rate	87%	82%
Pupa mortality rate	18%	0%
Total mortality rate	89%	82%
Second replicate		
Larvae mortality rate	80%	76%
Pupa mortality rate	50%	21%
Total mortality rate	90%	81%
Total		
Larvae mortality rate	85%	79%
Pupa mortality rate	39%	12%
Total mortality rate	90%	81%

Conclusions

Captive breeding has been proposed as a valuable conservation tool for invertebrates (Lewis and Thomas 2001). In this experiment, Common Mormon butterflies, *Papilio polytes*, were reared under captivity. The results support the hypothesis that the number of offspring from mimetic *P. polytes* females is smaller than the offspring from their non-mimetic counterparts before reaching the adulthood. Although the log-rank test for survivorship and the Welch test for the number of eggs laid, number of eggs hatched, and adult lifespan showed no significant difference between the two groups, the actual number of successfully metamorphosed larvae recorded was higher in the non-mimetic Common Mormon females. The larval developmental time mimetic *P. polytes* females was greater than that of the offspring from non-mimetic females. In addition, the Welch test for the cumulative number of deaths showed that the offspring from non-mimetic females have a significantly lower number of deaths. Factors affecting larval performance include host plants, human disturbance, temperature and humidity, and intraspecific competition. The larval duration in this study was longer than those reported from Malaysia, Pakistan, and India possibly because of different diets and climatic regimes.

Predation is reported to be the main reason for mortality in both larvae and adult stages for the Common Mormon butterfly in the wild. Female-biased predation in the wild is believed to be the reason female-limited mimicry exists. The non-mimetic females are preferentially attacked by predators as compared

to the males and mimetic females when the model species is present (Ohsaki 1995). In the wild, the natural predators of the Common Mormon usually learn to avoid *P. polytes* after an uncomfortable encounter with its unpalatable model, the Common Rose, *Pachliopta aristolochiae*. Therefore, habitat overlap with the model species is essential for the mimetic wing pattern to be effective (Uésugi 1996). Previous research on the mimetic and non-mimetic ratio in a population of *P. polytes* shows that the ratio depends on the abundance of Common Rose (Tsurui-Sato et al. 2019). With increasing populations of the *P. polytes* mimics, the defensive mimicry benefits decrease and the predation risk for the model species increases (Tsurui-Sato et al. 2019, Sato et al., 2020). In some cases, when the Common Rose model is not available, *P. polytes* females will have other species of butterfly, such as *Byasa alcinous* (Klug, 1836), as their mimetic model and increase their likelihood of survival (Katoh et al. 2017).

The data herein presented suggest that there is a trade-off in physiology with individuals carrying the Batesian mimicry gene having fewer individuals successfully surviving to the adult stage. Since this study is based on a strain created from only three parental individuals, it is possible that the results were affected by genetic factors other than the mimicry gene. However, if the main factor leading the study result is the Batesian mimicry gene, the cost would lead to higher mortality with individuals carrying the mimetic gene when there are no predators present. Conversely, based on previous research, when there are predators in the environment, the female-limited mimicry of the Common Mormon, *P. polytes*, serves as an aposematic signal to predators increasing the mimics' survival rate and, under appropriate mimic to model ratios, outweighing the mimics' physiological costs. In future work, the limitations of this study, such as the relatively small number of pairings and generations, the breeding environment, the inability to distinguish the male genotypes, and whether the physiological effects can be an adaptation should be addressed, first in the laboratory setting and then in field studies.

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Appendix

In this section, I include a few examples of the butterflies I have reared emphasizing some of the materials I use. Please, feel free to contact me if you have specific questions about rearing butterflies.

Figure A1. Two mimetic adult females *Papilio polytes* Linnaeus, 1758 (Papilionidae).

Figure A2. Two adult *Papilio polytes* mating. A mimetic female is on top.

Figure A3. Two *Graphium agamenmnon* (Linnaeus, 1758) (Papilionidae) larvae (final, fifth instar, on left, fourth instar on right). Note leaf margin feeding (arrow) on *Magnolia alba* leaf.

Figure A4. A recently emerged male *Graphium agamenmnon*. Note long setae in hind wing that characterize this species.

Figure A5. Fifth and last instar of *Papilio bianor* Cramer, 1777 (Papilionidae). Note leaf margin feeding (arrow) on *Citrus limon*.

Figure A6. Prepupa of *Papilio bianor* on *Citrus limon*. Note floral water tube, used to extend the life of cut plants.

Figure A7. Pupa of *Papilio bianor* on *Citrus limon*.

Figure A8. Adult female *Papilio bianor*.