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Alcohol content in fruit-feeding Lepidoptera: preferences and effects on flight time, pattern, and speed

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ABSTRACT

Fruit-feeding Lepidoptera live and feed on rotting fruit and tree sap flows. Eating fermented food is expected to alter their flight behavior making them sluggish and thus more susceptible to predation (Young 1979). Rotting food sources have similar chemical compositions but in different proportions. Ethanol is present in rotting fruits and sap flows (Omura and Honda 2003) and is most likely the agent causing sluggish or groggy behavior (Young 1979). In this study, I examine preference of fruit feeding *Caligo spp*. (Nyphalidae: Brassolininae) on different baits of varying ethanol concentrations. Furthermore, I examine whether alcohol content and time spent feeding on fruit impacts potential for predation, measured here as flight time, distance and the sporadic nature of the flight. This study was performed using existing fruit feeding platform in a large butterfly garden in Monteverde, Costa Rica. Small amounts of guaro: water solutions were added to rotting fruits at 10, 20, 30 and 35% ethanol. *Caligo spp.* individuals in the garden were allowed to choose among seven feeding stations, including three with no treatments (just rotting fruit). Butterflies having fed for different amounts of time were poked to see if they would fly. I found that *Caligos spp*. showed no preference for alcohol content, feeding freely on all fruit platforms. Behavior was influence by alcohol, but sluggishness was a function of time spent feeding, rather than alcohol content. Flight speed slowed as feeding time increased, regardless of alcohol content, suggesting that negative effects of ethanol were a function of feeding time rather than alcohol content in the fruit. The butterflies that drank shorter times flew longer. Still, there were no significant flight impairments from ethanol intake meaning that butterflies are adapted to deal with and use ethanol to their benefit. This was suggested by the fact that there were no significant differences in flight speed even for those butterflies feeding for over 90 minutes. As the highest feeding times in this study were only slightly greater than an hour and a half, this may have been too little time to impair flight. Stupor is widely reported in *Caligo spp*. and individuals on feeders in the morning were lying on their sides and very sluggish. These observations suggest that eventually, with more prolonged feeding times, ethanol will negatively impact flight and thus make the butterflies more susceptible to predation.

RESUMEN

Algunas mariposas viven y se alimentan de frutos maduros y savia de los árboles. Al alimentarse de comida fermentada se espera que se afecte el comportamiento de vuelo y este se vuelva aletargado y así más susceptibles a depredación (Young, 1979). Fuentes alimenticias maduras tienen composición química similar pero en diferentes proporciones. Etanol está presente en frutos maduros y savia (Omura and Honda 2003) y es el mayor causante del letargo (Young 1979). En este estudio yo examiné la preferencia de la mariposa frugívora *Caligo spp*. (Nyphalidae: Brassolinae) en diferentes sebos variando la concentración de etanol. Además examiné como el contenido de alcohol y el tiempo de vuelo tienen un impacto potencial en depredación, medido aquí como el tiempo de vuelo, distancia y la naturaleza esporádica del vuelo. Este estudio se realizó utilizando plataformas de alimentación en un mariposa rió en Monteverde, Costa Rica. Pequeñas cantidades de guaro: agua se añadieron a frutas maduras en concentraciones de 0, 10, 20, 30 y 35% de etanol. Los individuos de Caligo en el mariposario tenían 6 diferentes estaciones de alimentación para escoger, incluida una sin tratamiento (solo fruta madura). Las mariposas que se alimentaron en diferentes tiempos fueron tocados para ver si podían volar. Encontré que las mariposas no muestran preferencia por el contenido de alcohol en todas las plataformas de alimentación. El comportamiento fue influenciado por el alcohol, pero el letargo fue en función del tiempo de duración de la alimentación más que por el contenido de alcohol. La velocidad del vuelo disminuye con el aumento en el período de alimentación, sin importar el contenido de alcohol, sugiriendo un efecto negativo del etanol es en función del tiempo de alimentación más que el alcohol contenido en la fruta. Las mariposas que toman menos tiempo vuelan más. Aunque, no hay deterioro en el vuelo por el alcohol mismo esto significa que las mariposas están adaptadas para usar el etanol para su beneficio. El tiempo mayor de alimentación durante este estudio fue solo un poco mayor de una hora y media, este puede ser un período corto de tiempo para deteriorar el vuelo. Letargo ha sido ampliamente reportado para esta especie de mariposa y los individuos en comederos durante la mañana caen y son muy lentos. Estas observaciones sugieren que eventualmente, con tiempos de alimentación prolongados, el etanol puede afectar negativamente el vuelo y hacerlas así más susceptibles a la depredación.

INTRODUCTION

The family Nymphalidae includes three subfamilies of fruit-feeding butterflies, Satyrinae, Brassolinae, and Morphinae, all of which feed on rotting fruit and sap flows (Young 1979). Microbial fermentation products in sap flow, including alcohols, ketols, and carboxylic acids, stimulate foraging behavior in fruit-feeding butterflies (Omura and Honda 2003). Butterflies at rotting fruit or sap flows are often more approachable than would normally be the case (Goode 1999) and exhibit slow, erratic flight (Young and Muyshondt 1973). This has created the idea that alcohol byproducts of fermentation are causing butterflies to become inebriated. If so, this would increase the likelihood of predation, as these butterflies are palatable (Goode 1999). It has been hypothesized that the evolution of crypsis and prominent eye spots in these species is a response to the unusual sluggishness that results from their diet (Young 1979).

Saprophagous, or fruit-feeding, butterflies showed a clear preference for naturally rotting fruit sources over artificial ones made by mixing different fresh fruits with rum (Young 1972). In a study to determine exactly what components are responsible for feeding responsiveness, fruit-feeding butterflies did not show positive feeding responses to any one ingredient in isolation but preferred a combination of chemical compounds, most notably sugar, glycerin, ethanol and acetic acid (Omura and Honda 2003). While they prefer mixtures with all four components, there are large differences in the relative amounts of each component in rotting fruits and sap flows. For example, figs, persimmons, and bananas differ in that rotting bananas have the highest concentration of ethanol and rotting persimmons the highest concentration of sugars, glycerin and acetic acid (Omura and Honda 2003). This suggests that butterflies may choose rotting fruits based on the amounts of each of these potential components. Bananas, the fruit with the highest concentration of ethanol, are one of the most popular fruits fed upon by tropical butterflies (Omura and Honda 2003). Therefore, it could be that fruit-feeding butterflies prefer ethanol, despite possible inebriation and concurrent predation risk.

The present study first aims to explore whether fruit-feeding butterflies of the genus *Caligo* (subfamily Brassolinae, species C. *atrefus, C. brasilencis, and C. telamonius memnon*) prefer certain alcohol contents in the fruit they choose. Higher alcohol may mean an easier time feeding, as the fruit has been converted to fluid and butterflies are only able to feed on liquid since they use a proboscis, a straw-like structure (Omura and Honda 2003). On the other hand, high ethanol may increase the risk of predation. The second aim is to determine whether responsiveness and flight speed and pattern are affected by diet choice and feeding behavior. The likelihood of alcohol-related escape impairment increases not only with alcohol content but time spent feeding. As the *Caligos spp*. feed for a longer time, it is proposed they will be more affected by the ethanol and thus they will be less responsive and fly at a slower speed for less time before their next perch.

MATERIALS AND METHODS

This study was done at the Selvatura Butterfly Garden in Andunte Park, in Monteverde, Costa Rica. Seven feeding stations were used, each housing a plate of fruit consisting of bananas, papaya, mango, and pineapple, all in similar proportions. They were changed every Tuesday and Thursday by the Selvatura staff and replaced with overly ripe, slightly rotten fruit.

Alcohol preference

Every morning from 8:30 - 9 AM, the number of *Caligos spp.* feeding at each station was recorded. After this, alcohol solutions were measured out into containers. The 10% alcohol solution consisted of 14.3 mL of guaro (35% alcohol content) and 35.7 mL of water. The 20% alcohol solution consisted of 28.6 mL of Guaro and 21.4 mL of water. The 30% alcohol solution consisted of 42.9 mL of Guaro and 7.1 mL of water. The 35% alcohol solution consisted of 50 mL of guaro. After the addition of just enough fluid to wet the fruit (no puddling), the feeding plates were jostled so that all of the butterflies flew away. Feeders one, six, and seven were also observed, but no supplemental alcohol solution was added to them. Likewise, these control stations were jostled until all of the butterflies had flown away. From 9:30 - 10 AM, all seven feeders were visited again and the number of *Caligos spp.* present and feeding was recorded. At this point, each butterfly was carefully examined and location on the plate was noted. This way, it was easy to tell if the butterflies remained there the next time the feeder was visited.

Flight patterns and responsiveness

At each station, a subgroup of the butterflies that had been feeding for one half hour were tested for feeding position, responsiveness, and flight time, pattern, and speed, while others were left to continue to feed. Approximately one third of the butterflies that had known feeding times were tested at this point. First it was noted and recorded whether the ones being tested were standing to feed or leaning on their body or side. As an indication of responsiveness, approach distance was tested, a measurement of the necessary proximity to the butterfly before it flew away. The approach distances were designated as "near," defined as butterfly flight upon observer approach, "touch," where it was necessary to touch the butterfly for it to fly, or "pick-up," where the butterfly needed to be physically removed from the plate in order to fly. Flight time was recorded as the time the individual left the plate until landing. Flight pattern was recorded as "erratic," the normal pattern at which a *Caligos spp.* flew, or "slightly less erratic," and finally, flight speed, was recorded as "fast" or "medium." "Fast" was the speed at which *Caligos spp*. normally flew and "medium" was a speed slightly slower than that.

During the time intervals of 10 - 10:30 AM and 10:30 - 11 AM, the number of *Caligos spp*. were recorded at each feeder and the same set of tests was performed on select *Caligos spp.* They were performed on approximately half of the individuals that had been previously recorded as having started to feed and were still feeding 45 to 60 minutes later and those that were still feeding 90 minutes or more later. In other words, the butterflies tested were those for which feeding times were known and became progressively longer.

Rotating feeder treatments

In order to ensure that feeder placement (its location near a specific tree, its accessibility, its attention from probing tourists, etc.) was not affecting the butterflies' choice to feed from that particular station, treatment solutions were rotated into different feeders. On one occasion after the fruit was changed, for two consecutive days, station six received the 30% solution formerly at station four, while station seven received the 35% solution formerly at station five. On another occasion, for two days, station two received the 35% solution and station five received the 10% solution.

RESULTS

Feeding Preferences

For the duration of the study, there were no significant differences in the average number of butterflies observed at each feeder upon my arrival to the garden at 8:30 AM (Figure 1a., Friedman test, $x^2 = 3.93$, $p = 0.415$, df = 4). These butterflies were left with whatever food they were given the previous day; thus, they had a great deal of time to choose and settle on a feeder. Similarly, there were no significant differences in the average number of butterflies observed at each feeder one half hour after alcohol solutions were added (Figure 1b., Friedman test, $x^2 = 1.60$, $p = 0.809$, df = 4). There were also no significant differences the next times the feeders were observed, about an hour after the alcohol had been added (Figure 1c., Friedman test, $x^2 = 2.16$, $p = 0.706$, df = 4). Finally, there were no significant differences observed during the last time each feeder was counted, about 1½ hours after alcohol was added (Figure 1d., Friedman test, $x^2 = 5.72$, $p = 0.221$, $df = 4$). Also, from the four graphs collectively, there is no visible pattern showing that one feeder is more popular, suggesting there was no preference for particular alcohol content.

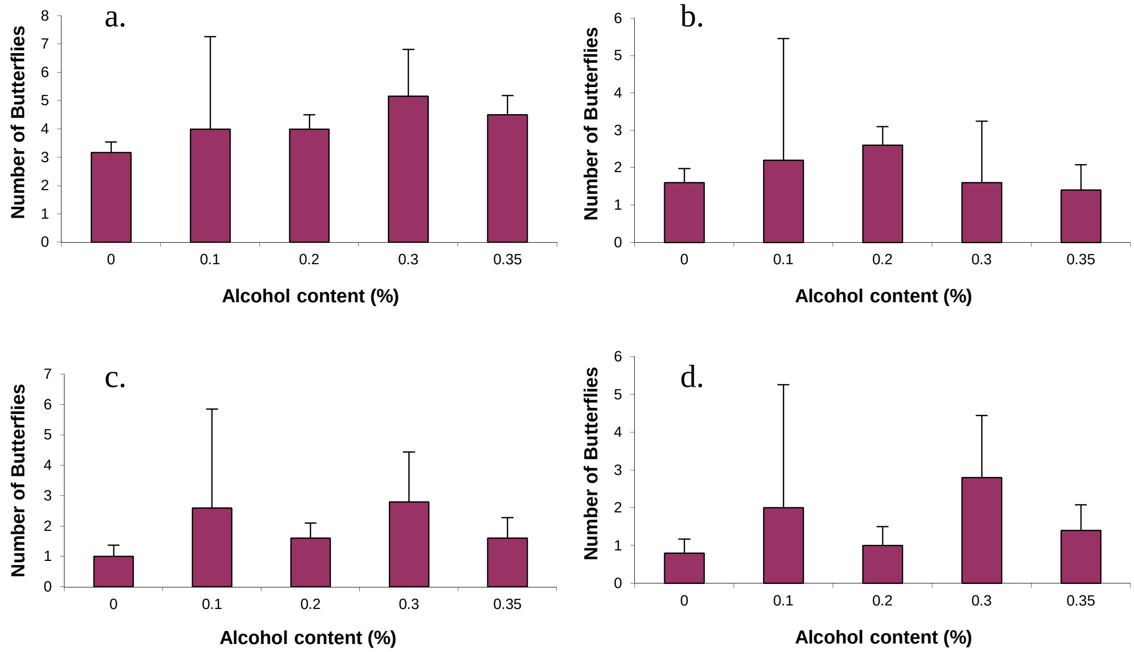

Figure 1.a. Average number of individual butterflies over the duration of the study visiting each feeder before alcohol was added that day (±SE, N = 30). These observations assume the alcohol content in each feeder is from the solution added the day before. Feeder with zero percent was the control, which had no solution added. b. Average number of individual butterflies over the duration of the study visiting each feeder one half hour after alcohol was added (±SE, N = 25). c. Average number of individual butterflies over the duration of the study visiting each feeder one hour after alcohol was added (±SE, N = 25). d. Average number of individuals over the duration of the study visiting each feeder 90 minutes after alcohol was added (±SE, N = 25).

Effects of Alcohol on Flight

a. Feeding position

Figure 6. Most butterflies fed standing up, but some, especially those found feeding for prolonged periods, were found on their sides.

The majority of the butterflies fed standing upright, but there was a good portion that fed while lying on their side or leaning on their body; however, there were no significant differences in drinking position among butterflies that fed for different amounts of time (Figure 6, Table 1, $x^2 = 4.34$, $p = 0.114$, $df = 2$). The butterflies found feeding in the morning, having been feeding on alcohol added the day before, were found mostly on their sides (Table 2). Although exact feeding time was unknown, it is likely that they had fed for many hours during the night and early morning.

Table 1. Number of butterflies that were found upright and leaning on their underbody or their side as a function of how long they spent feeding. Data shows no significant relationship between feeding position and length of time spent feeding.

Time spent feeding (minutes)	Upright	Leaning
15-30	20	1
45-60	11	4
90+	19	7

Table 2. Butterflies that were found at the feeders in the morning, having been feeding on alcohol added the day before, were found mainly leaning on their sides. This suggests that with prolonged feeding time, ethanol causes impairments in flight.

Time spent feeding (minutes)	Upright	Leaning
Overnight	2	11

b. Responsiveness and flight time

Almost every butterfly had to be physically touched or picked up in order for it to fly away (87%). Once they took off, there were no statistically significant effects on flight time, but there were important trends. Butterflies remaining at feeding stations for 15 – 30 minutes displayed a positive trend between time flown and alcohol content consumed; as the alcohol content in the feeder increased, the flight time increased as well (Figure 2a., Spearman rank correlation, Rho = 0.420, n = 21, p = 0.060). For those remaining at the feeders for 45 – 60 minutes, there was also a positive trend between flight time and alcohol content (Figure 2b, Spearman rank correlation, Rho = 0.363, n = 15, p = 0.174). For butterflies remaining at the feeders for more than 90 minutes, there was a negative trend in that as alcohol content increased, the flight time decreased (Figure 2c., Spearman rank correlation, Rho = 0.078, n = 26, p = 0.697). With one outlier, which consumed fruit devoid of alcohol, this group had flight times approximately equal to or shorter than the group that fed for 45-60 minutes suggesting no real difference due to feeding time.

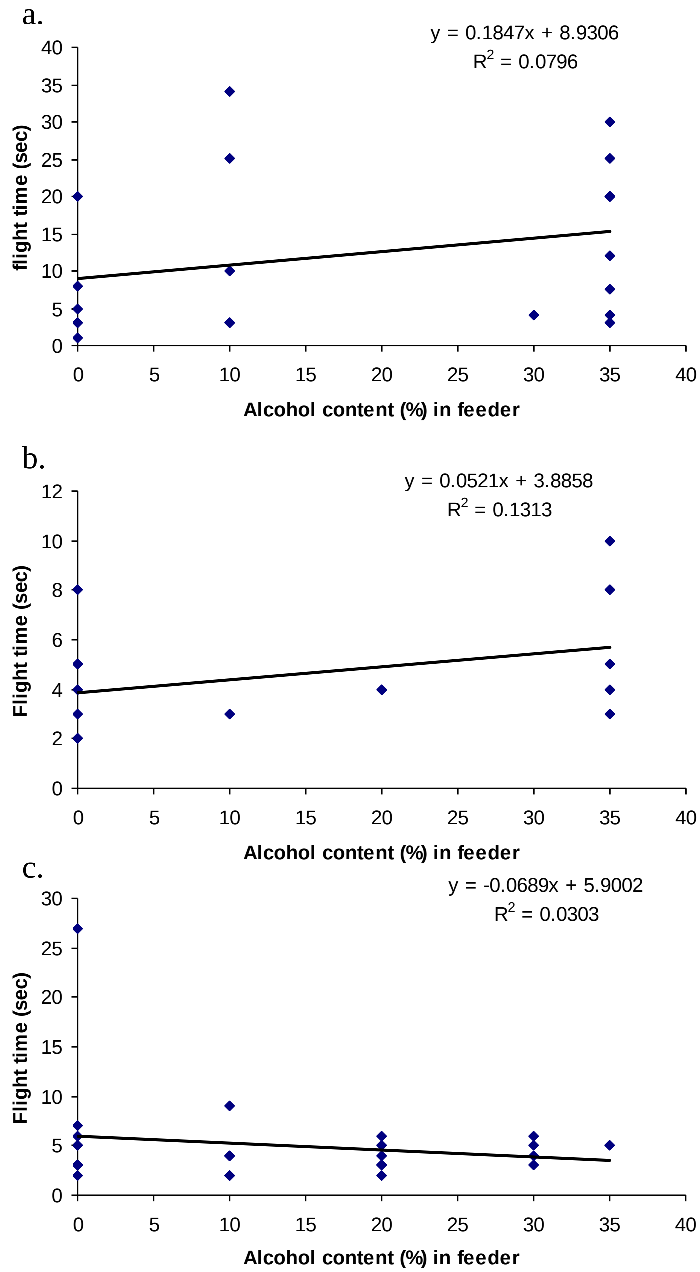

Figure 2. a. Regression line for butterflies at feeders for 15 –30 minutes (p = 0.215, N = 21). As alcohol content in the feeders increased, flight time increased. b. Regression line for butterflies at feeders for 45 – 60 minutes (p = 0.184, N = 15). As alcohol content in the feeder increased, flight time increased. c. Regression line shown for butterflies at feeders for over 90 minutes (p = 0.395, N = 25). As alcohol content in the feeder increased, flight time decreased

c. Flight pattern

There were no significant differences in flight patterns among butterflies that fed for different amounts of time. Nearly all (89%) of the butterflies flew in an erratic pattern like that of normal *Caligos spp*. and there were no trends based on flight time (Table 3, $x^2 = 2.533$, $p = 0.281$, $df = 2$).

Table 3. Number of butterflies that flew in erratic patterns and in slightly less erratic patterns as a function of how long they spent feeding. Data shows no significant relationship between flight pattern and length of time spent feeding.

Time spent feeding (minutes)	Erratic	Slightly less erratic
15-30	18	3
45-60	15	0
90+	22	4

d. Flight speed

Flight speed depended on length of feeding time; butterflies that fed for 15-30 minutes were, in general, faster than those that fed for 90 minutes or more. The time spent feeding by butterflies that are medium and fast fliers was not explained by chance; rather, fast butterflies were disproportionately often in the shortest feeding interval. In addition, the butterflies that flew at medium speeds were disproportionately often in the longest feeding interval (Table 4, $x^2 = 11.032$, $p = 0.004$, $df = 2$).

Table 4. Number of butterflies that flew at medium and fast speeds as a function of how long they spent feeding. Butterflies that fed for 15 – 30 minutes flew faster than expected in the null hypothesis and butterflies that flew for more than 90 minutes flew slower than expected. Results had a significant p-value. In the 15 – 30 minute group, more butterflies flew fast and fewer butterflies flew at a medium speed than the expected value predicted. In the 90+ minutes group, more butterflies flew at a medium speed than the expected value predicted.

Time spent feeding (minutes)	Medium	Fast
15-30	4	17
45-60	9	6
90+	17	9

DISCUSSION

In nature, saprophagous butterflies feed on low-reward, widely scattered food with little sugar, protein, or alcohol. Thus, they can not afford to be picky in which foods they choose to eat (Omura and Honda 2003). This explains why butterflies showed no preference for feeders. In order to have the energy necessary to fly for long periods of time, the butterflies, especially those as large as *Caligos spp.*, must drink for long periods of time; thus their bodies are probably adapted to deal with sustained ethanol intake. In fact, butterflies are not bothered by high amounts of ethanol as chemical analysis of the juice of rotting *Musa sapientum* revealed that there is only 2.6% ethanol content in natural rotting fruit (Omura and Honda 2003). The solutions used in this experiment raised the ethanol content of the fruit by large percentages to a maximum of 35%, yet the butterflies ate indiscriminately owing to the fact that they are not picky when feeding.

If fruit-feeding butterflies have, indeed, evolved without a preference for any specific alcohol content, it may be the case that eating sessions of lengths less than 90 minutes do not significantly impair flight. It appears these butterflies have a tremendous physiological ability to imbibe and metabolize the ethanol. In fact, their bodies have adapted to use the synergic effects of fermentation to their benefit. A previous study confirmed that fecundity, longevity, and egg size were shown to be highest in butterflies that were fed fruit over those that were fed sugar solution with lipids, yeast, or ethanol. There is not one pivotal substance that responsible for reproductive success, but rather, the combination of many substances found in fermenting foods. Another way these butterflies have adapted to and benefit from fermentation is that another study confirmed that ethanol may actually be a source of energy for the butterflies (Bauerfeind et al. 2007). This ability to metabolize and use the ethanol explains the positive trends present in the 15-30 and 45-60 minute feeding intervals. In these feeding times, higher ethanol levels did not impair them at all, but rather seemed to help them.

More time, however, means some flight impairment. Flight speed was significantly affected as the butterflies that fed for more than 90 minutes flew at slower speeds. This could have to do with the fact that the stomachs of the butterflies were just really full and therefore were not able to fly at normal speed. The idea that impairment is due to the fact that they are full and not that they drank too much ethanol is corroborated by the data that the time spent flying was not significantly affected by ethanol levels even in those butterflies that fed for 90 minutes or more. The negative trend suggests, however, that the higher ethanol contents might have significant impairing effects when feeding for even longer periods of time. We might have seen significantly different results to show the true impairment effects of alcohol on flight if there were butterflies feeding for longer time periods.

The relationship between feeding position and feeding time was also not significantly supported in feeding sessions of up to 90 minutes. In general, butterflies tended to stand while drinking; a position probably favored in nature since it allows the butterfly to take flight rapidly in the face of danger and allows maximum display of protective resemblance markings. When feeding time was very extensive, however, the butterflies did rest their bodies on the plate. The occasions I most frequently observed butterflies lying on their sides were when I arrived at the Garden in the morning and noted the number of butterflies at each station, which had been feeding without interruptions during early morning hours when Brassolinae butterflies are most active (DeVries 1987).

One area that will not be affected regardless of how long the butterflies feed for is pattern. There were no significant differences between length of feeding time and flight pattern. Erratic flight is an evolved and fixed behavior that does not change based on alcohol intake or feeding time. The individuals noted to have less erratic flight were those that only flew for short time periods (like a few seconds) and therefore it appeared as though they were flying in a less erratic pattern; however, if they had flown for longer, their pattern would have most likely been described as erratic. A study done on *Morphos peleides limpida*, confirmed that all adults, both male and female, are highly mobile and have irregular flight patterns (Young and Muyshondt 1973).

Fruit-feeders tend to live in dense forests, where fruit and sap are abundant but not visually apparent; therefore, they must use odor as opposed to color, as their cue to find food. Fermentation and the odors released provide cues from great distances to fruit-feeding butterflies that there is available food; food is not chosen based on nutritional value. These cues increase as the level of decay increases. Consequently, fruit-feeding butterflies have evolved to be attracted to and benefit from the ethanol produced in decaying fruit (Molleman et al. 2005). This highlights the effectiveness of eyespots as cryptic resemblance that successfully protects these butterflies from predators through deception and diversion of bites to the outer portion of the wings. In fact, one hypothesis has been proposed that relates feeding behavior to protective resemblance. It suggests that eyespots are most prevalent in those subfamilies that feed on ground-laying rotting fruit, which typically have the highest alcohol content. Eyespots are less prevalent on fruit feeders that feed arboreally, since the alcohol content of a fruit still hanging from a tree is less, and least prevalent in non-fruit-feeders (Young 1979). Given a widely-scattered, low quality resource, it seems the best the butterflies can do is eat what they encounter, eat a lot of it and stay close to it, so long as it is adequate, and subsequently deal with the effects of inebriation or become to full to fly at maximum speed and distance. It would be beneficial for future studies to investigate the weight of these butterflies as they feed to see if flight impairment is simply due to overeating. Also, future investigations should examine the metabolism of fruit-feeding butterflies to look for the presence of alcohol dehydrogenate, a compound that breaks down alcohol, to further learn about the effects of ethanol on their bodies.

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