The Effect of Tyrosine on Wing Patterns of the Cabbage White Butterfly (Pieris rapae)

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Reader(s): [Signature] 5/10/2019

Certified by: [Signature] Director, Honors Program

Date: [Signature]
The Effect of Tyrosine on Wing Patterns of the Cabbage White Butterfly (*Pieris rapae*)

A Thesis

Presented to the Department of Biological Sciences

College of Liberal Arts and Sciences

and

The Honors Program

of

Butler University

In Partial Fulfillment

of the Requirements for Graduation Honors

Katelyn Glaenzer

May 8, 2019
Introduction

1. Phenotypic plasticity

Phenotypic plasticity is when organisms with a single genotype produce different morphologies, physiological states, or behaviors in direct response to environmental stimuli (West-Eberhard, 1989). The environment is therefore important to both the determination of the phenotypes of organisms and to the fitness of organisms (Scheiner, 1993). The degree of interaction between the phenotype and the environment is called the reaction norm (Scheiner, 1993). The reaction norm, a plot with environmental variation (e.g. temperature) on the x-axis and phenotype on the y-axis, represents the range of possible phenotypes from a specific genotype due to interactions with the environment (Nijhout, 2003). Traits that do not change with environmental variation have flat reaction norms, whereas traits that do change have non-flat reaction norms, which could be linear, curved, or very complex shapes. While no organism is completely resistant to environmental effects on development, the sensitivity of particular traits to the environment is variable, i.e. some traits are more sensitive than others (Nijhout, 2003). Furthermore, while not all plasticity is adaptive, in variable environments, certain phenotypes may be more fit than others, depending upon specific details of each environments (Via et al., 1995). Therefore, the ability to express environment-dependent phenotypes is one of the most powerful means of adaptation used in nature (DeWitt et al., 2004). Although often adaptive, there are costs and limits to adaptive plasticity, which are difficult to distinguish and isolate from each other (DeWitt and Scheiner, 2004; Auld et al., 2009). Without an understanding of not only the benefits, but also the limits and costs, it is hard to understand the scope of the complexity of the plasticity (Auld et al., 2009).
2. **Seasonal polyphenism (i.e. seasonal patterns of predictable phenotypic plasticity)**

Phenotypic plasticity that has a predictable annual or seasonal pattern of variation is called seasonal polyphenism (Shapiro, 1976). As the seasons change, so does the temperature and day-length (photoperiod), along with other aspects of the physical and biotic environment. These changes may affect the fitness of the organisms but may also be the environmental cues that induce plasticity. It is interesting to note the environmental cues that induce the plasticity are not always the same as the environmental factor which directly affects the resulting phenotype through natural selection (Nijhout, 2003). For example, a common seasonal polyphenism is adaptation to cold stress or nutrient stress (Nijhout, 2003). However, the environmental cue which induces the adaptive phenotype may be a change in photoperiod, which is a consistent indicator of time-of-year (Nijhout, 2003).

Seasonal polyphenism is seen in organisms ranging from plants to insects (Shapiro, 1976). For example, water striders were found to be seasonally polyphenic for wing length in response to be both the stability of their water source and photoperiod, and in some specific cases the temperature as well. The water striders who must over-winter and create the initial breeding colony at each water source in the spring have long wings. However, following generations at each water source can have a variety of wing lengths. If the colony is located on a stable water source, such as a large lake without any danger of desiccation, the descendants will have short wings and be incapable of flying until the temperatures drop again. However, if the colony is located on an unstable water source, the population will be more dimorphic including both long and short wings, with the ratio of each depending on the degree of isolation from other water sources and populations (Shapiro, 1976).
Butterflies are a commonly used model organism for seasonal polyphenism because many species exhibit quantifiable seasonal variation in a variety of traits, such as generation time, growth rate, and final weight (Nylin et al., 1989; Mellström et al., 2010). They also show visible changes in wing patterns seasonally through multiple generations (Shapiro, 1976). As most butterflies are multivoltine species, meaning they have several distinct generations per year, the generations experience different environments over the seasons. This allows each generation to develop a phenotype most suitable for each season (Nijhout, 2003). The variation in wing patterns is thought to affect the organism’s thermoregulation (Shapiro, 1976). Each species’ phenotypic response is determined through temperature, photoperiod effects, or diet, with variation on the importance of each of those cues (Shapiro, 1976). It has been found that many butterflies have a specific period during development, usually in the late larval or early pupal stage, in which they are especially sensitive to the environmental conditions which affects their final plastic phenotype (Shapiro, 1976).

Pierid butterflies (Family Pieridae) have been extensively studied to better understand seasonal polyphenism, as they have several traits which may be associated with thermoregulation (Kingsolver and Wiernasz, 1991; Stoehr and Goux, 2008; Stoehr, 2010). As in other butterflies, the wing patterns of pierid butterflies are created through a series of scales which overlap and create a larger image. In pierids, a majority of the scales are un-melanized creating a light yellow to white color, but there are distinct black patches made of melanized scales (Stoehr, 2010). In the common small Cabbage White butterfly (Pieris rapae), there is a black patch on the tip of the dorsal forewing, called the apex (Figure 1). In addition to the apex on the dorsal forewing, there are two central spots (an anterior spot, DSpot 1 and a posterior spot, DSpot 2), as well as a collection of black scales on the basal portion of the discal cell of the wing. These regions have
been found to be phenotypically plastic (Stoehr and Wojan, 2016). The spots are also present on the ventral surface of the forewing (VSpot 1 and 2). The hindwing also has several wing pattern traits. The dorsal side of the hindwing has a spot akin to an apex (HS, meaning hindspot), as well as variation similar to that of the discal cell on the forewing (DHW, for dorsal hindwing). The ventral hindwing has variation in melanized scales similar to that on the dorsal side of the hindwing (VHW, for ventral hindwing) (Figure 1). There is a sexual dimorphism in Cabbage White Butterflies. Females generally have more prominent melanization of the discal cell and posterior dorsal forewing spots (DSpot 2). Males tend to have less melanization in these regions.

These wing pattern traits exhibit predictable seasonal changes, some of which have very specific functions in the fitness of pierid butterflies. The black scales allow the butterfly to absorb more sunlight, which allows it to warm faster and reach a higher body temperature (Kingsolver, 1985). This suggests that it is more important for butterflies developing in cooler temperatures, such as spring and fall, to have more black scales, at least on certain parts of the wings (Stoehr, 2010; Stoehr and Goux, 2008). Mark-recapture field experiments, combined with experimental manipulation of wing patterns shows that wing pattern variation has fitness consequences (Kingsolver, 1995). Most studies have shown that environmental temperature experienced during development (i.e. late larval, early pupal periods) has the greatest effect on wing pattern melanization, although photoperiod during development also seems to matter to some degree, at least in some species (Shapiro, 1976; Kingsolver and Wiernasz, 1991; Stoehr and Wojan, 2016). Another possible environmental cue that could affect plasticity, but that has not been investigated, is diet and dietary content.
3. Why nutrient variation?

As larvae (i.e. caterpillars), butterflies eat plants, referred to as their hostplants. Nutrients available to larvae vary seasonally because the hostplants vary seasonally, both in terms of the species present and the nutrients most abundant in a given species (Slansky, 1982; Rodrigues and Moreira, 2004). This seasonal variation in hostplant nutrient content has been seen to produce plasticity in other species of insects. A specific example of an organism using diet as a plasticity cue is the *Nemoria* caterpillar which is found in the southwest United States. *Nemoria* caterpillars develop a polyphenism to mimic the fundamental plants in their diets (Greene, 1989). Summer generations develop to mimic oak twigs whereas spring generations mimic oak catkins (Greene, 1989). The amount of tannins in the oak leaves varies seasonally, and it is the tannin variation that influences what body form the caterpillars will develop. Diet polyphenism is also exhibited by male dung beetles. The size of male horns is a form of sexual selection, but it was found to also be affected by the quality of the diet that is available to the males (Emlen, 1997). Males raised on high-quality diets were larger than those on a low-quality diet, but also had disproportionately larger horns (Emlen, 1997). With this knowledge that other insects use diet as an environmental cue, I hypothesized that it would matter in pierids too. Nutrient variation could serve as an informative predictor of future conditions, therefore contributing to the adaptiveness of plasticity or nutrient variation could just be a problem that must be dealt with, therefore limiting the ability of the butterflies to optimally match phenotype to seasonal temperature variation.

The black pigment in the dark scales of the Cabbage White Butterfly is melanin. Melanin is created through a complex pathway that is metabolically costly for most butterflies (Futahashi and Fujiwara, 2005). Melanin is synthesized from the amino acid tyrosine (Sugarmaran, 2002),
which must be acquired from the hostplant. Tyrosine and other tyrosine derivatives are not just important in the melanin pathway, but also integral in immune defense, wound healing, cuticle hardening, and egg tanning (Sugumaran, 2002). Studies have found with increasing certainty that melanin production is costly (Stoehr, 2006; Lee et al., 2008; Ma et al., 2008). The potential for tyrosine-based variation in the melanin-based phenotypes of other butterflies (Futahashi and Fujiwara, 2005) led me to hypothesize that variation in wing pattern in Cabbage White Butterflies could be due to dietary nutrient variation, i.e. variation in dietary tyrosine. This hypothesis predicts that the melanin-based wing pattern traits will differ between butterflies raised on normal compared to tyrosine-supplemented diets, with tyrosine supplementation increasing melanization of (at least some) wing pattern traits.

4. Tyrosine supplementation and tyrosine-based trade offs

As discussed above, under cool (spring or fall) conditions, Cabbage White Butterflies have more black scales in some wing areas and this is likely to be adaptive for effective thermoregulation. In particular, the increased cool-season melanization occurs in basal dorsal surfaces (discal cell region) and on the ventral hindwing. However, this variation does not account for the entire phenotypic plasticity of the wing pattern in Cabbage White Butterflies. Interestingly, the other wing pattern traits (apex and spots) show the opposite pattern, as those traits are less melanized (i.e. smaller) in the cool season and largest in the middle of summer. These two different and opposing seasonal patterns result in negative correlations between the traits known to be involved in thermoregulation (basal portions and ventral hindwing) and these other traits (apex and spots) (Figure 2). These traits, i.e. apex and spots, are probably not involved in thermoregulation directly, but instead might be reduced in the spring and fall because they compete with the other traits (i.e. basal dorsal surfaces and ventral hindwing) for the
tyrosine necessary for melanin production. In other words, the negative correlation between the apex and spots, on the one hand, and the basal dorsal surfaces and ventral hindwing, on the other, might be because the butterflies cannot maximally melanize all these traits on limited tyrosine. This trade off hypothesis predicts that tyrosine supplementation will eliminate or reduce the strength of the negative relationship between the melanin-based traits in these two trait groups. In other words, with sufficient tyrosine the butterflies should be able to melanize the basal dorsal wing surfaces and ventral hindwing while also being able to produce large spots and apexes.

To test these predictions, I raised Cabbage White Butterflies on an artificial diet that contained all nutrients Cabbage White Butterflies have been found to need (Webb and Shelton, 1988) as a control diet (Table 1). Tyrosine was added to this diet, at 3 grams per batch, with all other ingredients remaining the same, to create an experimental high-tyrosine diet. Butterflies were reared under two temperature-photoperiod combinations (cool, short-day and warm, long-day) that mimic seasonal extremes. These temperature/day-length treatments were used simply to create a high degree of wing pattern variation (Stoehr and Wojan, 2016), thereby increasing the variation in allocation to wing pattern traits the butterflies were forced to make. I then quantified the extent of variation in melanization (i.e. the size) of the wing pattern traits from digital photographs to evaluate my predictions. Hereafter, larger traits or greater area of black scales is synonymous with “greater melanization”.

**Methods**

**Rearing**

Eggs were collected from a laboratory population of *P. rapae* which were derived laboratory stock (Carolina Biological Supply). Control diet was added to small plastic cups, and an average of five eggs per cup were added before hatching. Once the caterpillars had hatched,
fed and reached 1cm, they were randomly placed singly (i.e. one per cup) on new fresh diet of either the control or the high-tyrosine type. Once they were placed on their diet, they were randomly placed in incubators (either cool, short-day or warm, long-day) for them to develop. Approximately 600 larvae were assigned to each diet treatment. The cool, short-day (spring and fall imitation) incubators were set to 19°C with 8 hours of light and 16 hours of darkness. The warm, long day (summer imitation incubators) were set to 25°C with 16 hours of light and 8 hours of dark. The warm, long-day incubator butterflies took around one week to emerge from their pupae, whereas the cool, short-day butterflies took about two weeks. After emerging, each butterfly in the experiment was transferred into small plastic envelopes and immediately frozen to preserve their wings. Of the approximately 1200 butterflies reared, 444 females and 556 males survived to emergence and had wings that could be photographed. Sample sizes vary somewhat in different analyses because in some cases some, but not all, traits on a wing could be measured. For example, folds or stains on wings sometimes prevented me from being able to measure a trait.

Measurement

After the butterflies were frozen, they were examined to determine their sex and numbered. Females have consistently larger secondary spots on their dorsal forewings (DSpot 2). Their wings were then prepared for photographing by cutting the wings from the abdomen taking great care as to not damage them. They were digitally photographed and the photos were entered into ImageJ, a software that analyses the area covered by black scales, in mm², in specific wing locations. ImageJ is able to count each scale below a color threshold on the specified wing location. Therefore, I was able to determine the exact amount of melanization of those regions. There are six locations on the dorsal side of the wing of interest, a black patch at the tip of the
wing, termed the apex, as well as two central spots (DSpot1 and DSpot2), as well as the basal portion of the discal cell of the dorsal forewing (bDFW) (Stoehr and Wojan, 2016). There is also a spot on the leading edge of the dorsal surface of the hindwing (HS, for hind spot) and a polygon of the lower part of the wing on the hindwing (DHW) which I measured. The ventral side of the wing has three areas of focus, two spots on the forewing (VSpot1 and VSpot2), and a polygon of melanized scales on the ventral hindwing (VHW) (Figure 1).

Data Analysis

The results of all experiments were analyzed using JMP 14.1.1 statistical software (SAS Institute Inc., Cary, North Carolina). Because larger butterflies are expected to have larger wing pattern traits, I controlled for the size of the butterfly by dividing the area of the trait by the total size of the wing. I conducted two sets of analyses to evaluate the two different predictions. In one set of analyses, I compared the size of each trait using a series of t-tests to determine if there were differences between these traits for butterflies reared on the control and higher tyrosine diets. Sexes were analyzed separately because of strong sexual dimorphism in wing pattern traits.

In a second set of analyses to evaluate the prediction of the tradeoff hypothesis, I created two “trait groups” to represent the traits involved in the trade off. One group consisted of the traits that are more melanized in the cool seasons and are thought to function in thermoregulation: this group was the sum of the areas of the discal cell region, the hindwing (DHW) on the dorsal wing, and the hindwing melanization (VHW) on the ventral side. This measurement is referred to as the “thermoregulation group”. The other group consisted of the sum of the areas of the spots on the dorsal forewing (DSpot 1 and DSpot 2), the apex, the spot on the hindwing (HS), and the spots on the ventral forewing (VSpot 1 and VSpot 2). This group is referred to as the “apex/spots Group” and includes the traits that are less melanized in the cool
season. The apex/spot groups for both male and female butterflies were normally distributed, while the thermoregulation group was right skewed and so was normalized through a log transformation for both sexes. Separate ANCOVAs for each sex were run with diet as the categorical independent variable, the thermoregulatory group trait as the continuous independent covariate, and the size of the apex/spot group as the dependent variable. This test was used to test the tradeoff hypothesis: if the negative relationship (i.e. negative slope) between the apex/spot group and the thermoregulation group is reduced (or eliminated) by supplemental tyrosine, the hypothesis is supported (Figure 2). Differences between the slopes of each treatment would provide support for the hypothesis and would be indicated by a significant diet by thermoregulation group interaction.

**Results**

Using t-tests, I compared the wing pattern traits Cabbage White Butterflies on the high tyrosine and control diets (Tables 2 and 3). In female butterflies, five of the nine measured traits were significantly more melanized in the butterflies fed the high-tyrosine diet compared to the control diet. These were the apex (p=0.01), HS (p=0.002), discal cell (p=0.006), DHW patch (p=0.002), and VHW patch (p=0.044). The other female traits were larger (or similar: VSpot 2) in high-tyrosine butterflies as well, but these differences were not significant. In male butterflies, the apex (p=0.003) and Dspot2 (p=0.022) were significantly larger in animals raised on the high-tyrosine diet, compared to the controls, but the Vspot2 (p=0.009) was larger in butterflies fed the control diet. The apex and the Dspot2 are more melanized in the higher tyrosine diets, however the Vspot2 is more melanized in the control diet for male Cabbage White Butterflies. The other male traits were not significantly different, although of the remaining six all but one (VSpot 1) was larger in the high-tyrosine butterflies.
The ANCOVA for female butterflies found a significant negative relationship between the thermoregulation group and the apex/spot group \((F_{1,443}=6.3994, p=0.0118)\), which was expected given what is already known about how those traits vary seasonally and how temperature and day-length affect them. The high tyrosine diet increased the melanization of the apex/spot group \((F_{1,443}=8.2146, p=0.0044)\), which was also expected given the results of the t-tests. A regression line was created for each diet between the apex/spot group and the thermoregulation group. The slope of the regression line for the high tyrosine treatment was less negative (less steep) than that of the control treatment, but this interaction was not quite statistically significant at the 0.05 level \((F_{1,443}=2.9950, p=0.0842)\) (Figure 4). The ANCOVA for male butterflies found similar results to the females, with both the negative correlation between the categories \((F_{1,555}=101.9422, p<0.0001)\) and the effect of diet (high tyrosine=more melanization) on the apex/spots group \((F_{1,555}=9.5087, p=0.0021)\) being significant. However, the slopes of the two regression lines were very similar and thus the interaction was not significant \((F_{1,555}=0.2898, p=0.5906)\) (Figure 4).

**Discussion**

The results of this study are generally consistent with the hypothesis that some variation in melanin-based wing traits is due to dietary tyrosine content because we observed an increase in the melanization of many traits due to increased tyrosine in the diet. We found that five traits are significantly more melanized for females on the high tyrosine diet. The other four traits in females were also larger on the high tyrosine diet, although those differences were not statistically significant. In males, the effects were far less pronounced. The apex and dorsal posterior spot was significantly larger in the high tyrosine butterflies, but the ventral posterior spot was smaller in the high tyrosine butterflies. Some of the non-significant differences in males
were in the direction of more melanization in high tyrosine butterflies, but others were not. Taken together, these findings suggest that female wing patterns may be more sensitive to dietary tyrosine than male wing patterns.

The increase in tyrosine seems to particularly effect the thermoregulation traits for the females, with all three traits (discal, DHW, and VHW) being significant for the higher tyrosine diet. The apex is significantly larger for the higher tyrosine group in both males and females (Table 2 and 3). The males do not show as much of an effect for wing traits using the t-tests (Table 3). Lack of tyrosine sensitivity of the apex and spot group traits could be due to their role in sexual communication (Stoehr and Wojan, 2016). Female Cabbage White Butterflies are more sensitive to the dietary changes than males. One hypothesis might be that due to the sexual dimorphism, these traits are more sensitive to dietary-based tradeoffs because melanin is under more demand. Their apex, spots, and discal regions are larger, meaning that there could be more competing demands for melanin among female traits than that for males. More competing demands for melanin means that they could be more sensitive to the amount of tyrosine present in their diet. Their wing traits require more melanin than males, but this might not be limited to just wing pattern traits. For example, eggs require resources so maybe some of the tyrosine is going into eggs (Jones et al., 1982).

The tradeoff hypothesis is not strongly supported, due to the lack of a significant effect of tyrosine supplementation on the negative relationship between the apex/spot group wing traits and the thermoregulation group wing traits. The negative correlation between wing traits is not affected for the males (Figure 4) and is only modestly (and not quite significantly) affected for females (Figure 3). In other words, the negative correlation that was predicted to level off with the addition of tyrosine was not affected (Figure 2). Perhaps Cabbage White Butterflies do not
have the ability to flexibly reallocate melanin to different traits, even with excess tyrosine. The tradeoff hypothesis assumes that such a tradeoff is plastic. It is possible that the negative correlation between these trait groups is a tradeoff that is based on a long evolutionary history of limited resources. In that case, the genome is expected to evolve negative genetic correlations among traits that trade off, but these genetic correlations will be “hard wired” in place, *i.e.* will no longer be sensitive to resource variation. In general, it is recognized that herbivorous insects such as butterflies are protein-limited (Slansky, 1982), but whether this applies to tyrosine, and to this species, is not known.

In this study, butterflies were only used from a lab reared population. The effects of dietary tyrosine that were found could be different for wild butterflies than lab reared butterflies. Future work on the effect of diet on Cabbage White Butterflies could examine a variation in total nutrient availability (*i.e.* not just tyrosine) in their diet. This could determine if diet influences the development of their wing pattern at all, or if the vast majority of seasonal wing pattern variation is due to seasonal variation in temperature and photoperiod. Understanding the relative importance of different cues to wing pattern traits will increase our understanding of the complexity of phenotypic plasticity that some insects are capable of. Other plasticity cues should be explored for each species, as each cue and the interaction of cues creates the final complex phenotype.

**Acknowledgments**

I thank Dr. Andrew Stoehr for his support, use of his lab, and help with the research. I also thank Dr. Shelley Etnier for her help in improving the manuscript.
Figures and Tables

Figure 1: Focal wing pattern traits of the Cabbage White butterfly. Dorsal (A) and ventral (B) wing pattern traits are visible. Forewings are on top, hindwings on bottom. Characteristic one is the apex and characteristics two and three are the dorsal spots (DSpot 1 and DSpot 2). Characteristic four is the discal cell, characteristic five is the dorsal hindwing (DHW), and characteristic six the spot on the leading edge of the dorsal surface (HS). On the ventral wing, characteristics seven and eight are the ventral spots (VSpot 1 and VSpot 2), characteristic nine is the ventral hindwing (VHW).
**Figure 2: The tradeoff hypothesis prediction.** The dashed line represents the negative relationship between two trait groups observed in Cabbage White Butterflies in the wild, and under control dietary conditions. The black line represents the predicted weakened negative relationship between these traits with the increase in dietary tyrosine. With sufficient tyrosine, the butterflies are predicted to maintain large apex/spot size even with heavily melanized thermoregulation group traits.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tr>
<td>Wheat Germ</td>
<td>60 g</td>
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<tr>
<td>Cabbage Flour</td>
<td>15g</td>
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<tr>
<td>Casein</td>
<td>27g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>24g</td>
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<tr>
<td>Beck’s salt mix</td>
<td>9g</td>
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<td>Torula yeast</td>
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<td>Flax Oil</td>
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<tr>
<td>Agar</td>
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Table 1: Ingredients used in creation of control diet. (Modified after Webb and Shelton, 1988)
Table 2: Female Cabbage White Butterflies wing pattern traits and dietary tyrosine. The significant differences are bolded.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean ± SEM</th>
<th>N</th>
<th>Mean ± SEM</th>
<th>N</th>
<th>t value</th>
<th>DF</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Apex</td>
<td>0.257 ± 0.005</td>
<td>188</td>
<td>0.241 ± 0.004</td>
<td>256</td>
<td>-2.57</td>
<td>380.25</td>
<td>0.0104</td>
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<td>DSpot 1</td>
<td>0.043±0.001</td>
<td>188</td>
<td>0.042±0.001</td>
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<td>-1.05</td>
<td>376.94</td>
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<td>DSpot 2</td>
<td>0.039±0.001</td>
<td>188</td>
<td>0.037±0.001</td>
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<td>-1.38</td>
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<td>Discal</td>
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<td>0.184±0.004</td>
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<td>400.81</td>
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<td>Hspot</td>
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<td>0.043±0.001</td>
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<td>0.033±0.001</td>
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<td>Log VHW</td>
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Table 3: Male Cabbage White Butterflies’ wing pattern traits and dietary tyrosine. The significant differences are bolded.

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<tr>
<th>Trait</th>
<th>Mean ± SEM</th>
<th>N</th>
<th>Mean ± SEM</th>
<th>N</th>
<th>t value</th>
<th>DF</th>
<th>P-value</th>
</tr>
</thead>
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<td>0.1723±0.003</td>
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<td>-2.381±0.037</td>
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<td>DSpot 2</td>
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<td>0.000±0.000</td>
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<td>-2.30</td>
<td>316.23</td>
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<td>Discal</td>
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<td>275</td>
<td>-1.942±0.024</td>
<td>281</td>
<td>-1.52</td>
<td>553.61</td>
<td>0.1298</td>
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<td>0.020±0.001</td>
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<td>-0.14</td>
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Figure 3: ANCOVA between the thermoregulation wing traits (log mm$^2$) and the spot wing traits (mm$^2$) with diet as the controlling variable for female Cabbage White Butterflies. High tyrosine (HT) and control diets are both present on the graph. The p-value for the effect of diet is 0.0044, for the relationship between the two wing trait categories is 0.0118, and for the interaction between the two is 0.0842.
Figure 4: ANCOVA between the thermoregulation wing traits (log mm$^2$) and the spot wing traits (mm$^2$) with diet as the controlling variable for male Cabbage White Butterflies. High tyrosine (HT) and control diets are both present on the graph. The p-value for the effect of diet is 0.0021, for the relationship between the two wing trait categories is less than 0.001, and for the interaction between the two is 0.5906.
References


