

**The effects of environmental disturbance during the developmental stages of Painted Lady
Butterflies (*Vanessa cardui*)**

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Abstract

The presence of humans has greatly altered the natural balance of countless ecosystems and has negatively impacted many individual organisms. Human activity within a natural habitat physically agitates the organisms that dwell there and can result in asymmetrical characteristics due to stress during their developmental periods. This study sought to examine the effects of stress disturbance during the developmental stages of Painted Lady butterflies (*Vanessa cardui*). A sample population of Painted Ladies was divided into three treatment groups: experiencing physical agitation during the larval stage, experiencing stress during the pupa stage, or not experiencing stress throughout their lifecycle. Once the butterflies emerged, forewings and hindwings were measured, wing color and pattern was examined, and wing deformations were noted. The data collected suggested that physical stress had no significant statistical effect on forewing or hindwing asymmetry, wing deformity, or time of emergence from the pupa. However, a relationship was suggested between wing deformity and wing color asymmetry in the treatment group subjected to stress during the chrysalis stage. Future research should further explore butterfly response to disturbance during their sensitive developmental periods in relation to asymmetrical wing color and deformation. With further scientific evidence, the detrimental effects of human presence in natural environments can be acknowledged and a movement towards conservation can be instigated.

Introduction

Environmental stresses present in a habitat affect the fitness of the organisms it supports in a variety of ways. Fitness can be measured by quantifying survival, reproductive success, community structure, species diversity, and relative abundance or density (Clarke 1995), but these variables can be extremely difficult and unrealistic to measure. Instead, fluctuating asymmetry can be used to indicate genetic or environmental stresses affecting an organism.

Fluctuating asymmetry is defined as random divergences from perfect symmetry in a bilaterally symmetrical physical trait (Lens et al. 1999). It is thought to be a result of an organism's inability to buffer against environmental disturbances (Santos 2001) and thus serves as an indicator of decreased fitness. From an evolutionary standpoint, individuals capable of withstanding various disturbances due to environmental stresses during development will be at a selective advantage (Lens et al. 1999) of developmental stability. Increased stress causes an individual to spend more energy to remain in a state of homeostasis and therefore forces the organism to exhibit developmental instability (Hendrickx et al. 2003). Developmental stability or instability directly reflects overall population quality (Windig et al. 2000); suggesting that stressed or low quality individuals should have lower developmental stability and thus leading to higher fluctuating asymmetry (Santos 2001).

Although there are some empirical contradictions in past research (Bjorksten et al. 2000), many researchers suggest that disturbances during the developmental stages of organisms can produce asymmetrical physical characteristics in the adult life stage (Clarke 1995). The existence of environmental stresses during development has been correlated with decreased developmental stability and therefore leading to increased fluctuating asymmetry in a variety of plants and animals (Wright and Zamudio 2002). The most abundant species are expected to

display the lowest amount of asymmetry and the most endangered species are expected to display the highest amounts of asymmetry (Windig et al. 2000), reflecting developmental stability.

Although past research has primarily focused on the effects of pollutants and extreme environmental stresses such as temperature (Hendrickx et al. 2003), actual physical agitation has not been accurately studied. Physical agitation is a likely event for an organism dwelling in an area with human presence. For Painted Lady butterflies (*Vanessa cardui*), human presence within a habitat can cause physical agitation to these vulnerable insects and thus is a common threat to their survival. This species is almost globally distributed, except in South America and the poles, and is migratory. In their pupa stage, they are immobile and cannot remove themselves from a stressed environment. Due to the butterfly's distinguished three-stage life cycle, researchers are allowed to focus on each developmental period to determine which is most sensitive. These fragile insects exhibit obvious bilateral symmetry throughout their lifecycle and can be closely examined for abnormalities.

This research is relevant to conservation biology because fluctuating asymmetry serves as a warning system for threatened ecosystems. Besides affecting the individual organism, asymmetry is related to mating success (Santos 2001), thus affecting populations as a whole.

This study attempts to determine if physical disturbance during the larval and pupa stages lead to asymmetrical characteristics in the adult stage. We predict that stress during the developmental periods of Painted Lady butterflies (*Vanessa cardui*) will lead to asymmetrical qualities in butterfly wing color and pattern, body measurements in their adult stage, and abnormal time of emergence from the pupa.

Methods

Inflicting Stress

A sample size of 30 Painted Lady butterflies were held in an isolated room with constant temperature, light cycles, and lacking physical disturbance. Each individual was placed within an individual plastic cup with a lid. The population was evenly divided into three treatment groups, of which the individuals were subjected to stress in the larval stage, pupa stage, or neither stage, serving as a control group. According to a random schedule, physical stress was inflicted daily by twenty firm shakes of the plastic cup, back and forth. The individuals were checked twice daily, once at noon and once at midnight. The date and time by which an individual had transformed into the next life stage was recorded and the length of each life stage was calculated. The time spent as a larva or pupa was categorized based on emergence at the time of observation, so that those emerging at noon qualified as 0.5 days and those emerging by midnight qualified as a full day. Deformations and other observations were recorded throughout the developmental stages as well as after emergence from the pupa as a butterfly.

Measuring Stress Response

Once the butterflies had emerged from their pupa, they were placed in the freezer overnight to kill them. Next, they were removed from the freezer and left at room temperature to thaw. Once thawed, the wings were spread and body parts moved into standard mounting placement. The prepared specimens were photographed using a Nikon D40 Digital SLR camera in an environment with standardized light, height, and angle. NIH Image software was then used to precisely measure the length of the forewing, hindwing, and body from tip of head to end of abdomen. Additionally, the symmetry of wing color and pattern was examined and compared.

Statistical Analysis

The forewing, hindwing, length of chrysalis stage, color asymmetry, and wing deformity data was uploaded to R Commander and various tests were performed in order to statistically analyze the effect of physical agitation. 1-way ANOVAs were used to determine the effect of treatment on forewing asymmetry, hindwing asymmetry, and length of chrysalis stage. Chi-square tests of independence were used to determine the relationship of wing deformity on color asymmetry and treatment on color asymmetry.

Results

Stress during developmental stages had no statistically significant effect on forewing asymmetry (1-way ANOVA, $F = 0.36$, $df = 2$, 25 , $p = 0.703$; Figure 1). Likewise, stress during developmental stages had no statistically significant effect on hindwing asymmetry (1-way ANOVA, $F = 1.08$, $df = 2$, 24 , $p = 0.356$; Figure 2).

Treatment group had no statistically significant effect on the occurrence of wing deformity (chi-square test of independence, $X^2 = 5.54$, $df = 2$, $p = 0.063$; Figure 3). Due to the relatively small sample size, these data must be considered cautiously. Nevertheless, butterflies subjected to stress during their pupa stage were 40% more likely to express wing deformations than those in the control group or those experiencing stress in the larval stage. Wing color asymmetry depended on the presence of wing deformity (chi-square test of independence, $X^2 = 8.40$, $df = 1$, $p = 0.004$; Figure 4). When deformed, 40% of the butterflies exhibited color asymmetry and when the butterflies were not deformed, 0% exhibited color asymmetry.

Finally, stress during different developmental stages had no statistically significant effect on length of time spent in the chrysalis (1-way ANOVA, $F = 0.70$, $df = 2$, 26 , $p = 0.505$; Figure 5). The rate of development was similar among the three treatment groups.

Discussion

The lack of statistically significant findings of this study suggest that fluctuating asymmetry is not be the best measure of the effect of environmental stress on Painted Lady butterflies. Although fluctuating asymmetry has been proposed to show relationships with stress, fitness, sexual selection, heterozygosity, and inbreeding, the idea lacks a reliable theoretical foundation (Bjorksten et al. 2000). Some of the inconsistencies in the studies of fluctuating asymmetry can be attributed to large sampling error, providing a poor estimate of latent developmental stability (Bjorksten et al. 2000). Because studies use a sample of two data points to estimate the variance in individual development (Bjorksten et al. 2000), inconsistent data is inescapable and is evident in countless studies (Bjorksten et al. 2000, Nijhout 1991, Windig et al. 2000, Clarke 1995).

On the other hand, many other researchers claim that although there are many inconsistencies in the field of study focusing on fluctuating asymmetry, it remains the ideal biomonitor to identify populations under stress, because of its apparent generality with respect to species, traits, and stress (Bjorksten et al. 2000). Due to its generalized application, fluctuating asymmetry remains a reliable predictor of individual performance (Bjorksten et al. 2000). While many studies have found substantial evidence that fluctuating asymmetry reflect levels of environmental stress, a number of studies have suggested that fluctuation asymmetry is most apparent in sexually selected traits (Bjorksten et al. 2000) or genetic stress (Kark et al. 2004, Lens et al. 1999).

Asymmetry in physical characteristics may remain masked by underlying genetic factors, as suggested by a number of researchers within their studies of fluctuating asymmetry. Studies found that increased asymmetry in the most disturbed environments may have resulted from

inbreeding depression or high levels of homozygosity (Lens et al. 1999). Inbreeding depression has been measured using reductions in fecundity, fertility, and genetic variation within a population and when utilizing these determining factors, a significant increase in asymmetry has been found as result of inbreeding (Clarke 1995). The Painted Lady butterflies used in this study were purchased from a small distributor in Bremerton, Washington, which thus introduces the likely possibility of small gene pools and inbreeding within their insect populations. Though the possibility of inbreeding exists, it is unlikely that the effect of genetic stress on fluctuating asymmetry could negate that of environmental stress to the point that it would entirely conceal a trend of developmental instability, resulting in a difference in levels of asymmetry (Kark et al. 2004).

Although there was no statistical significance suggesting the appearance of fluctuating asymmetry in physical characteristics of the butterflies as a response to physical stress, there was significant statistical evidence suggesting a relationship between wing deformity and color asymmetry. As environmental perturbations can be stressful to an organism's normal path of development (Clarke 1995), morphological traits are much more sensitive to developmental conditions than fluctuating asymmetry (Santos 2001). In further studies of fluctuating asymmetry, significant evidence has been found suggesting that the size of morphological traits is far more responsive to stress (Bjorksten et al. 2000). Despite the fact that pigments are not synthesized until just before adult emergence from the pupae, the fate of the scale cells is determined in the final larval stage and early pupae stage (Brakefield and Breuker 1996). There are three major processes of wing pattern formation: the determination of wing shapes, the arrangement of veins within the wings, and wing color pattern. Though all three stages take place in the larval stage (Kark et al. 2004), the pupa stage is suggested to be most vulnerable

(Nijhout 1999). This vulnerability is due to the fact that the caterpillar has been broken down into a liquid within the pupa and is undergoing an extreme reconstruction of its physical composition. In this study, the butterflies exhibiting morphological deformities most likely exhibited color asymmetry within their wings. Those that exhibited both of these interrelated characteristics most often formed into a pupa on the floor of their holding cup or detached from the roof of the cup during their pupa stage due to rigorous shaking. The infliction of stress disturbed the path of their normal development but had no effect of the rate of development as suggested by the lack of variation or statistical significance in time of emergence from the pupa.

Despite the discrepancies regarding fluctuating asymmetry, it is important to consider the implications of environmental stress upon an individual organism. Relationships between environmental stressors and developmental stability have been shown in a number of organisms (Clarke 1995). Energy becomes limiting due to a large amount allocated to stress defense, thus energy will be limited for both reproduction and developmental stability (Hendrickx et al. 2003). Alternative explanations to fluctuating asymmetry try to describe the increase in magnitude in fluctuating asymmetry and fitness relationships in the presence of environmental stress. These explanations assume that fluctuating asymmetry more accurately reflects developmental stability under stress and that elevated stress might strengthen the relationship between individual quality and fitness as low quality individuals are more likely to exhibit asymmetrical characteristics (Hendrickx et al. 2003 and Clarke 1995). Low quality individuals are more likely to be selected against and thus are eventually eliminated from the population (Hendrickx et al. 2003). Fluctuating asymmetry can serve as a relative estimator of stability, but there is no standard value or presence of asymmetry that indicates stability (Clarke 1995), consequently instigating such scientific discrepancies.

The issue with using fluctuating asymmetry to measure the effect of stress on quality is that the developmental and genetic foundation of fluctuating asymmetry is not fully understood (Bjorksten et al. 2000). Hopefully a pattern will emerge because the theory remains promising if a few changes occur in the methods for studying fluctuating asymmetry. In order to form better predictions about the expected levels of asymmetry and a function of selection, trait functionality, or physiology across the range of species, the particular life history, range-limiting factors, and spatial distribution of the species should be properly accounted for (Kark et al. 2004). Future research should use larger sample sizes in order to detect stress effects and increase genetic diversity within the sample sizes so that genetic factors, such as inbreeding, can be reduced. Maybe even multiple generations will be necessary to conclude the effect of stress on asymmetrical physical characteristics and the universal impact on fitness and developmental stability. With further research, the threshold of environmental stress on deformity for butterflies may be discovered and the error created by the existence of high levels of asymmetry even under optimal conditions (Clarke 1995) may be reduced. The continuing study of fluctuating asymmetry is promising and may present insight into the conservation of species subject to stress due to the destructiveness of human presence.

Literature Cited

- Bjorksten, T. A., K. Fowler, and A. Pomiankowski. 2000. What does sexual trait FA tell us about stress? *TREE* **15**:163-166.
- Brakefield, P. M. and C. J. Breuker. 1996. The genetical basis of fluctuating asymmetry for developmentally integrated traits in a butterfly eyespot pattern. *Biological Sciences* **263**:1557-1563.

- Clarke, G. M. 1995. Relationships between developmental stability and fitness: Application for conservation biology. *Conservation Biology* **9**:18-24.
- Hendrickx, F., J.-P. Maelfait, and L. Lens. 2003. Relationship between fluctuating asymmetry and fitness within and between stressed and unstressed populations of the wolf spider *Pirata piraticus*. *Journal of Evolutionary Biology* **16**:1270-1278.
- Kark, S., L. Lens, S. Van Dongen, and E. Schmidt. 2004. Asymmetry patterns across the distribution range: Does the species matter? *Biological Journal of the Linnean Society* **81**:313-324.
- Lens, L., S. Van Dongen, C. M. Wilder, T. M. Brooks, and E. Matthysen. 1999. Fluctuating asymmetry increases with habitat disturbance in seven bird species of fragmented afro-tropical forest. *Biological Sciences* **266**:1241-1246.
- Nihjout, H. F. 1991. *The Development and Evolution of Butterfly Wing Patterns*. Smithsonian Institution Press, Washington D.C.
- Santos, M. 2001. Fluctuating asymmetry is nongenetically related to mating success in *Drosophila buzzatii*. *Evolution* **55**:2248-2256.
- Windig, J. J., P. T. Rintamaki, A. Cassel, and S. Nylin. 2000. How useful is fluctuating asymmetry in rare and abundant *Coenonympha* butterflies. *Journal of Insect Conservation* **4**:253-261.
- Wright, A. N. and Zamundio, K. R. 2002. Color Pattern Asymmetry as a Correlate of Habitat Disturbance in Spotted Salamanders (*Ambystoma maculatum*). *Journal of Herpetology* **36**:129-133.

Tables and Figures

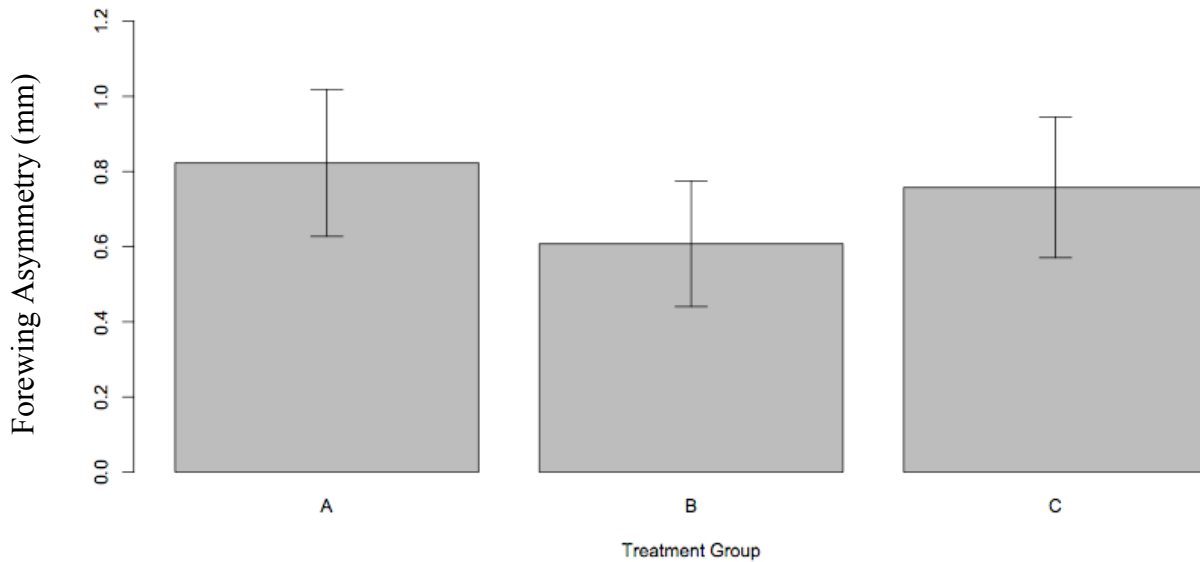


Figure 1. The effect of stress during developmental stages on asymmetry in mean forewing length (\pm SE). A sample size of 28 Painted Ladies (*Vanessa cardui*) was divided into three treatment groups: 10 experienced stress during the larval stage (A), 9 experienced stress during the pupa stage (B), and 9 experienced no stress throughout their lifecycle (C). Results suggest that physical agitation had no statistically significant effect on forewing asymmetry regardless of treatment group ($p = 0.703$).

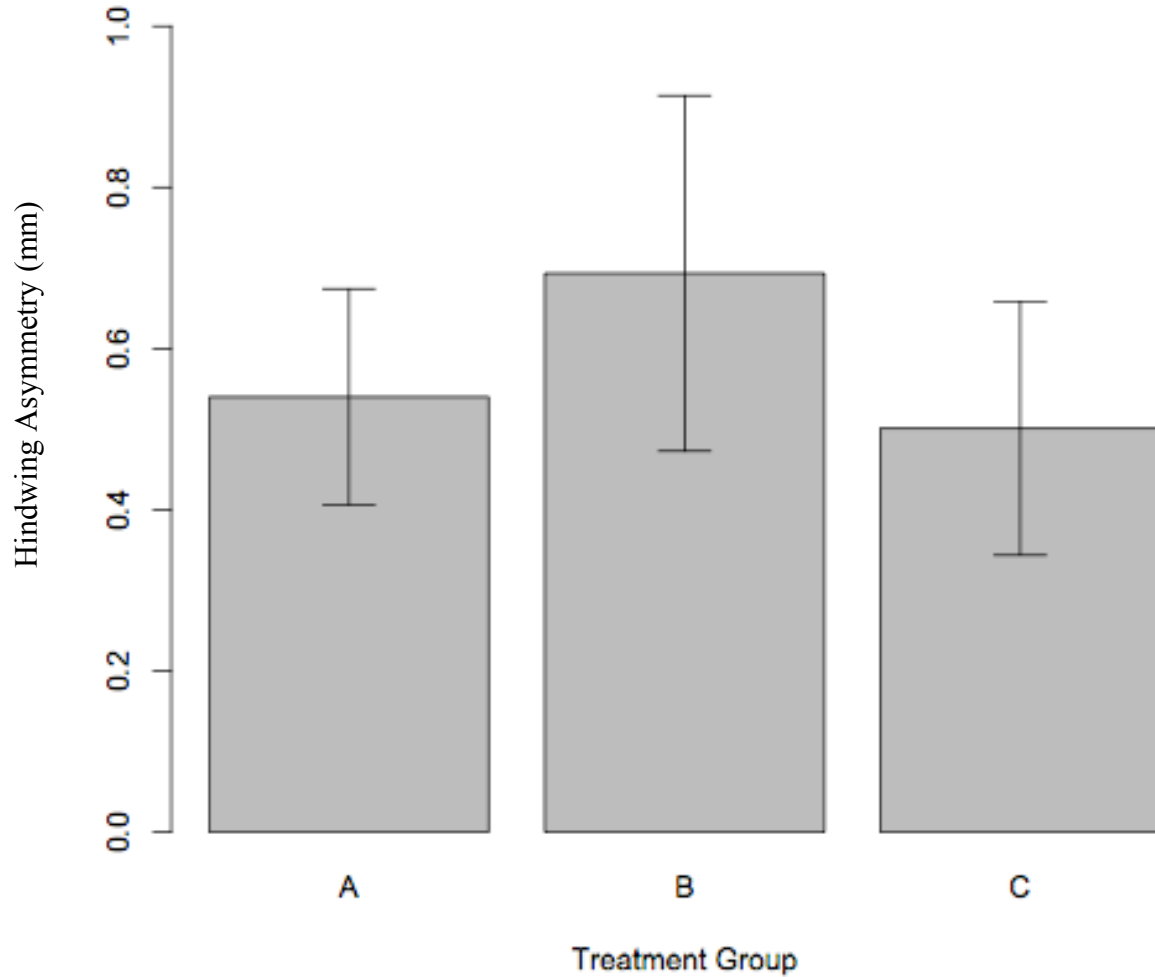


Figure 2. The effect of stress during developmental stages on asymmetry in mean hindwing length (\pm SE). A sample size of 28 Painted Ladies (*Vanessa cardui*) was divided into three treatment groups: 10 experienced stress during their larval stage (A), 9 experienced stress during their pupa stage (B), and 9 experienced no stress throughout their lifecycle (C). Results suggest that physical agitation had no statistically significant effect on hindwing asymmetry regardless of treatment group ($p = 0.356$).

Occurrence of Deformity

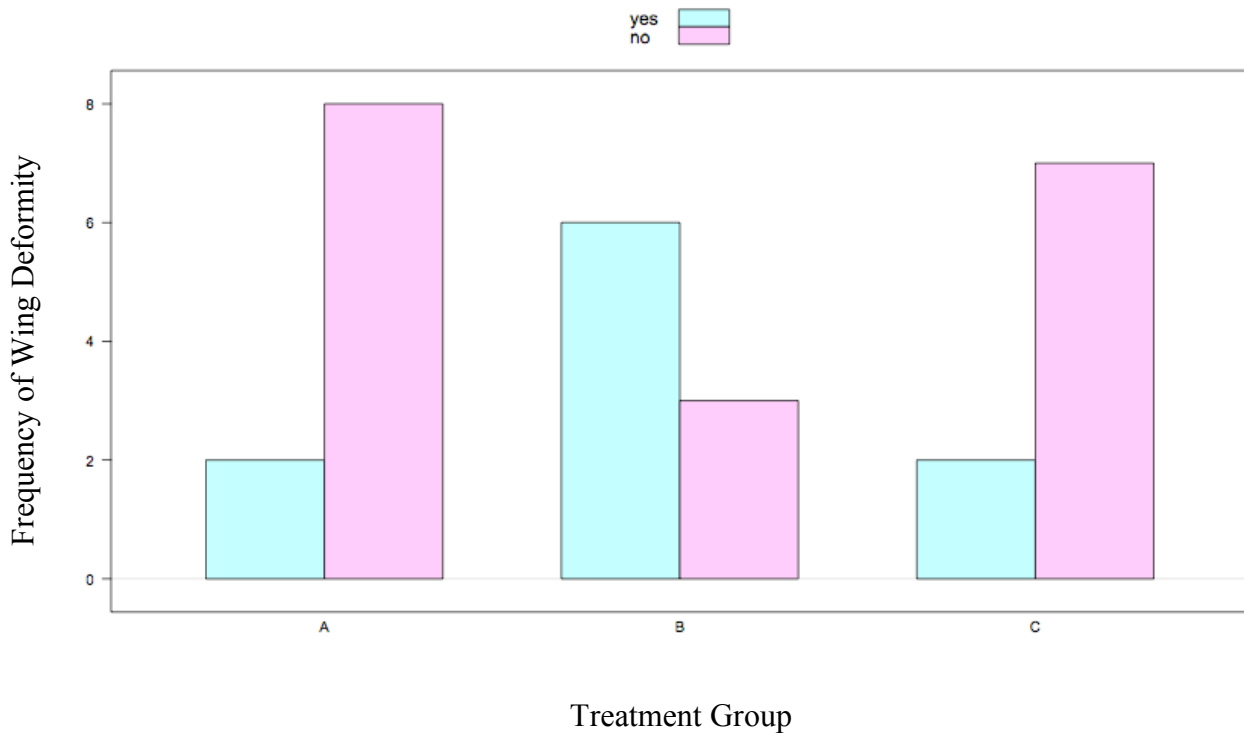
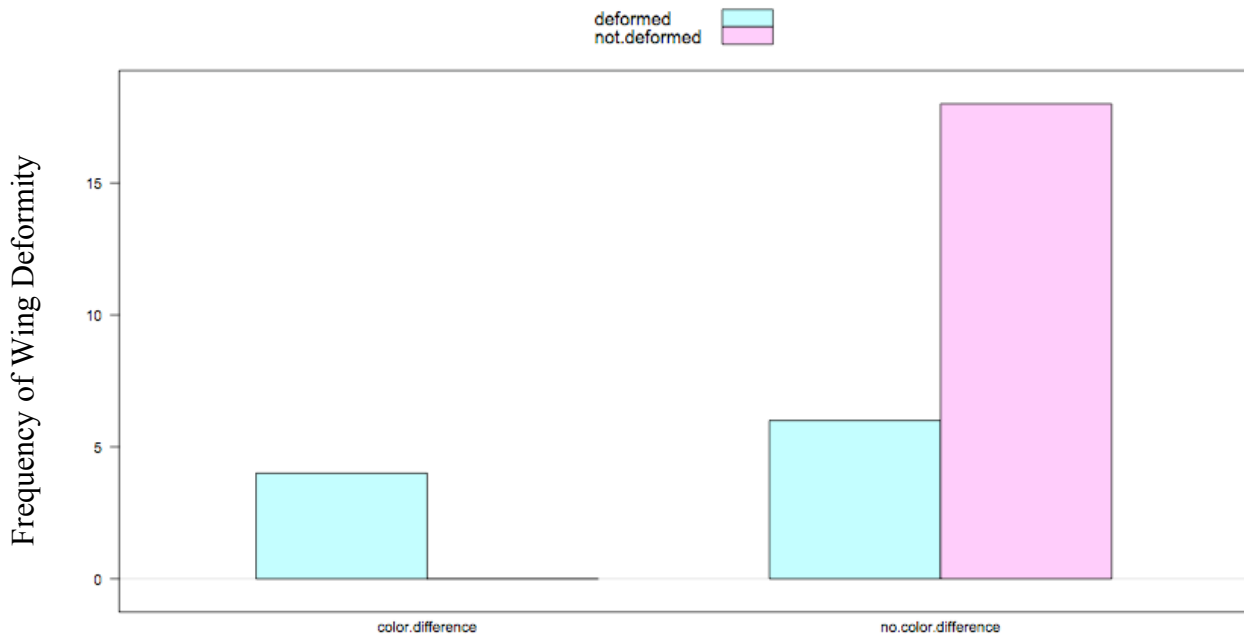


Figure 3. The relationship between stress and the occurrence of wing deformations within treatment groups of varying stages of butterfly development. A sample size of 28 Painted Ladies (*Vanessa cardui*) was divided into three treatment groups: 10 experienced stress during their larval stage (A), 9 experienced stress during their pupa stage (B), and 9 experienced no stress throughout their lifecycle (C). Results suggest that the effect of physical agitation and wing deformation act independently ($p = 0.063$). However, butterflies experiencing stress during their pupa stage were 40% more likely to express wing deformations than those in the control group or those experiencing stress in the larval stage.

Occurrence of Deformity



Presence of Color Asymmetry

Figure 4. The relationship between wing deformity and color asymmetry. A sample size of 28 Painted Ladies (*Vanessa cardui*) was divided into three treatment groups: 10 experienced stress during their larval stage (A), 9 experienced stress during their pupa stage (B), and 9 experienced no stress throughout their lifecycle (C). Results suggest that color asymmetry depended on wing deformity. When deformed, 40% exhibited color asymmetry and when not deformed, 0% exhibited color asymmetry.

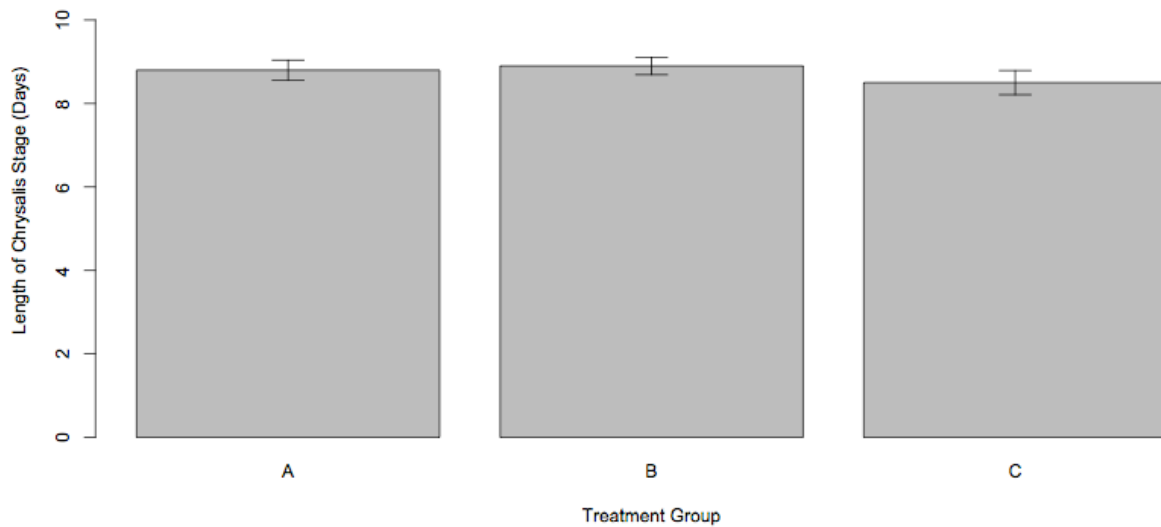


Figure 5. The effect of stress during developmental stages on mean time spent in the pupa stage (\pm SE). A sample size of 28 Painted Ladies (*Vanessa cardui*) was divided into three treatment groups: 10 experienced stress during their larval stage (A), 9 experienced stress during their pupa stage (B), and 9 experienced no stress throughout their lifecycle (C). Results suggest that physical agitation had no statistically significant effect on time of emergence from the chrysalis regardless of the treatment group ($p = 0.505$).

Appendix

A. R Commander Outputs:

Forewing I-Way ANOVA

```
> summary(AnovaModel.1)
      Df Sum Sq Mean Sq F value Pr(>F)
Treatment.Group 2 0.2277 0.11383 0.3574 0.703
Residuals      25 7.9615 0.31846
```

```
> numSummary(Dataset$Forewing.Difference..mm. ,
+ groups=Dataset$Treatment.Group, statistics=c("mean", "sd"))
      mean      sd data:n
A 0.8230000 0.6179185   10
B 0.6077778 0.5010932    9
C 0.7577778 0.5608426    9
```

Linear Hypotheses:

```
      Estimate Std. Error t value Pr(>|t|)
B - A == 0 -0.21522  0.25929 -0.830  0.688
C - A == 0 -0.06522  0.25929 -0.252  0.966
C - B == 0  0.15000  0.26602  0.564  0.840
(Adjusted p values reported -- single-step method)
```

Hindwing I-Way ANOVA

```
> summary(AnovaModel.7)
      Df Sum Sq Mean Sq F value Pr(>F)
Treatment.Group 2 0.4906 0.24530  1.079 0.3558
Residuals      24 5.4559 0.22733
1 observation deleted due to missingness
```

```
> numSummary(Dataset$Log.Hindwing , groups=Dataset$Treatment.Group,
+ statistics=c("mean", "sd"))
      mean      sd data:n data:NA
A -0.3435086 0.2533065    9    1
B -0.3130398 0.3788515    9    0
C -0.6130026 0.6886909    9    0
```

Linear Hypotheses:

```
      Estimate Std. Error t value Pr(>|t|)
B - A == 0  0.03047  0.22476  0.136  0.990
C - A == 0 -0.26949  0.22476 -1.199  0.465
C - B == 0 -0.29996  0.22476 -1.335  0.390
(Adjusted p values reported -- single-step method)
```

Deformity Chi-Square Test of Independence

Pearson's Chi-squared test

data: .Table
X-squared = 5.5447, df = 2, p-value = 0.06252

```
> .Test$expected # Expected Counts
  yes    no
A 3.571429 6.428571
B 3.214286 5.785714
C 3.214286 5.785714
```

Deformity-Color Chi-Square Test of Independence
Pearson's Chi-squared test

data: .Table
X-squared = 8.4, df = 1, p-value = 0.003752

```
> .Test$expected # Expected Counts
      deformed not.deformed
color.difference  1.428571  2.571429
no.color.difference 8.571429 15.428571
```

Time of Emergence 1-Way ANOVA

```
> summary(AnovaModel.10)
      Df Sum Sq Mean Sq F value Pr(>F)
Treatment.Group  2  0.8103  0.40517  0.7023 0.5046
Residuals      26 15.0000  0.57692
```

```
> numSummary(Dataset$Length.of.Chrysalis.Stage..Days. ,
+ groups=Dataset$Treatment.Group, statistics=c("mean", "sd"))
  mean    sd data:n
A  8.8 0.7527727  10
B  8.9 0.6582806  10
C  8.5 0.8660254   9
```

Linear Hypotheses:

```
      Estimate Std. Error t value Pr(>|t|)
B - A == 0  0.1000    0.3397  0.294  0.953
C - A == 0 -0.3000    0.3490 -0.860  0.670
C - B == 0 -0.4000    0.3490 -1.146  0.495
(Adjusted p values reported -- single-step method)
```