The Influence of the Eyespots of Peacock Butterfly (*Aglais io*) and Caterpillar on Predator Recognition

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**Abstract:**

The main purpose of this study is to verify or refute the famous existing theory that the eyespots found on the wings and the bodies of various insects are a kind of imitation which triggers birds, the predator of insects, to have a sense of avoidance by making them recognize the insects as their predator. The first experiment was conducted on the peacock butterfly using models with eyespots and those without eyespots. To reduce the gap between the model and real organism as much as possible, the method used in the prior experiment (Stevens et al. 2008) was adopted\([A3]\). A single butterfly model without eyespots was used as the control group, and a pair of a butterfly models with eyespots and another without eyespots was used as the treated group\([A4]\). We assumed that if the existing theory, imitating eyes, is correct, bird are unwilling to attack the model without eyespots in treated group because of the eyespots of peacock butterfly. The second experiment was conducted using caterpillar models with eyespots and those without eyespots arranged in the same way as the first experiment. According to the results of the experiment, it is difficult to conclude that the eyespots of peacock butterfly trigger a sense of avoidance for birds as there was no significant difference in the numbers of the attacked peacock butterfly models without eyespots between the control group and the treated group. The second experiment was conducted using caterpillar models with eyespots and those without eyespots arranged in the same way as the first experiment. As a first experiment, we assumed If the eyespots of caterpillars are imitating eyes of big predator, bird will be unwilling to attack the models without eyespots in treated group.
it located near the caterpillar model with eyespots. However, there was no statistically significant difference in the numbers of attacked caterpillar models without eyespots between the control group and the treated group. Thus, the second experiment shows that the caterpillar with eyespots does not imitate the eyes of the predator and it indirectly supports the findings of the first experiment. Through the results of the two experiments, it is possible to cast doubt about the existing theory that the eyespots actually imitate the eyes of the natural enemy of the predator.

Keywords: Eyespots, Imitation, Peacock butterfly, Caterpillar

Introduction

Research motive

In 1890, The numerous moths and butterflies which has eyelike patterns in the shape of a concentric circle on the wings was studied by Edward Poulton. Since then, he wrote on his book, the colours of animals, that the eyespots thwart the attack of birds, the natural enemy of insects, by triggering birds to recognize the eyespots of the insect as the eyes of their own natural enemy has been accepted without much doubt for more than a century.

While it has been proven that the eyespots of insects can defend themselves from the attack of their predator through various experiments (Vallin, Jakobsson et al, 2005), there is no sufficient proof that the reasons is that they imitate the eyes of a larger predator. Still, many researches have been carried out so far accepting the existing theory that the eyespots of the wings of insects imitate the eyes of the predator of birds (Stevens and Ruxton, 2014). For example, it is stated in the publication of Rota and Wagner (2006) that “it is indubitable that the eyespots on the wings of a giant silk moth imitate the eyes of a mammal predator”, but there was no sufficient ground suggested for support of such an assertion.

In his research (Stevens, 2005, Stevens et al, 2007), Stevens et al. showed that the mechanism of repelling the predator is not related to imitating the eyes of other big animals through the experiment of changing the pattern of the eyespots of the wings of peacock butterfly (Aglais io) into other shapes (a bar shape, triangle shape, etc.). Furthermore, he asserted that thwarting predation is related to the color contrast of the patterns. With his research, the verity of the existing theory began to be examined again. However, many researches conducted so far have focused on making new theories. As a result, controversies are still going on over the mechanism of avoiding predation, that is, if it is because of triggering the predator to has a sense of avoidance or not. Currently, there are many hypotheses about the role of the eyespots on the wings of insects including imitation of the eyes of the predator, disturbance, and causing fear with the unfamiliar pattern (Sebastiano De Bona et al, 2015), etc., and it is necessary to verify the hypotheses.
This study is focused on verifying the existing theory by preparing experimental grounds about the formation of a sense of avoidance for birds using the eyespots of peacock butterfly and caterpillar as used in preceding researches.

Research objective

To verify the existing theory that the insects defend themselves from their natural enemy by imitating the eyes of a larger predator using the eyespot pattern

Materials and methods

Theoretical background

There are several hypotheses about the real role of the eyespot pattern of insects: 1) The insects thwart the attack of the predator by imitating the eyes of a larger potential predator. 2) The insects prevent attack of the predator by visual disturbance. 3) The insects thwart predation by making the predator have a vague fear with a strange pattern (Sevastiano De Bona et al, 2015).

In his recent dissertation (2008), Stevens asserted that what triggers the phenomenon of avoidance of the predator is not eye imitation but color contrast. He performed the experiment using peacock butterfly models that he personally produced. The models were produced by printing the image of wings and attaching it to a dead mealworm. During the process of the experiment, he made variations by modifying the shapes of eyespots on the wings to a triangle and a bar and changing the color of the concentric circle, but there was no significant influence on predation. In doing so, he refuted the existing theory by showing that predation can be thwarted even if the pattern on the wings is not necessarily a circular shape similar to eyes. Instead, he demonstrated that the percentage of the peacock butterfly models attacked by the predator decreased when the size of eyespots and the color contrast between the eyespots and the wings was increased. However, the dissertation did not clearly explain how the color contrast worked on the vision of the birds, and no clear conclusion could be drawn as there was counterargument that it is possible that even the triangular or bar shapes can look like eyes.

Merilaita et al. (2011) made models with 4 dots and 2 dots by attaching dead mealworm to the wings of peacock butterfly. He thought that the models with 2 dots would be attacked less if the eyespots look like the eyes of a larger predator and the models with 4 dots would be attacked less if the color contrast has an influence. However, there was little difference in the results of the experiment between the two values and he suggested that a new theory would be necessary about the role of the eyespots.

Another research conducted by Hossie and Sherratt (2012, 2013) used caterpillar. They performed the experiment by making green artificial caterpillars. They confirmed that the survival rate of caterpillars increased when the head of the caterpillar and the eye shape grew bigger when a concentric circle pattern was drawn on the head of the caterpillar. In addition, they found out that the concentric circle was more effect when it was positioned on the head of the caterpillar than when it was on the body. Based on such a result, they asserted that the worm imitated a snake with its eyespots and supported the existing theory. They also supported the theory that the eyespots imitated dyes with the
fact that the effect of thwarting predation was greater when the eyespots moved like the frowning of a large animal. However, he also acknowledged the limitation of his research saying that it was not exactly certain that only one theory is correct.

Like this, controversy continues while a single theory is not adopted due to the researches suggesting various viewpoints. In this study, we thought that it was necessary to verify and refute the existing hypotheses instead of proposing a new hypothesis. We judged the existence of a sense of avoidance felt by birds by using the peacock butterfly and artificial caterpillar models that were used the most frequently for the experiment of insects in preceding researches. We designed the experiment supposing if birds have a sense of avoidance, the reason would be 'imitating eyes' as asserted in the existing theory, and on the contrary, it would be interpreted as 'inducing visual disturbance' if birds don't feel a sense of avoidance.

We thought it was difficult to verify theories exactly in the existing researches because they made new models that did not exist in nature. The researches lacked reliability generality of verification by assuming a situation that did not exist. To solve such a problem, this research aimed to draw the desirable results by using the models in the same way as they exist in nature unlike the existing research and just changing their number and arrangement.

In the experiment, a single model without a pattern was used as the control group, and a pair of a model with a pattern and another without a pattern was used as the treated group. If predation is thwarted by a sense of avoidance felt by birds due to the eyespots, predation of the model without a pattern put together with the model with a pattern in the treated group will be thwarted, too. However, if predation is thwarted due to another reason such as visual disturbance, the model without a pattern positioned around the model with eyespots will not be influenced much by predation.

**Experiment process**

Preparation of peacock butterfly and caterpillar models

We decided to make models for the experiment as it is difficult to collect live insects due to their characteristics moving in different places. To reduce the gap between the model and real organism as much as possible, we applied the method of producing peacock butterfly models used by Stevens et al. (2008), Merilaita et al. (2011) and many other dissertations, and applied the method of Hossie and Sherratt (2012, 2013) for production of artificial caterpillar models.

We produced peacock butterfly models by printing eye shapes on the white(#FFFFFF) waterproof paper, whose material quality is similar to that of the wings of butterflies and attaching it to the tree, etc. in the place of experiment and putting a dead mealworm in the center to provide a thing for the predator to eat. At this time, we made the size and the color of the wings and the background of all the models the same for control of variables. Using waterproof paper, we attached a specific pattern on the wings of peacock butterfly in the form of a sticker. Its size was 65mm in width and 32mm in length. The background color of the wings was grey(#808080) (The background color, too, may be a factor which affect the results of the experiment.).

The artificial caterpillar model is produced with a dough made by mixing flour and lard at a 3:1 ratio. Put 25ml of green(#008000) food coloring and 50ml
of water to 600g of dough and dye it to make caterpillars that look like real ones. The diameter of the dough is 5mm and its length is 15mm. The eyespot was made with yellow and black food coloring and the diameter was 2-3mm.

The experiment was conducted at a place off the hiking trail in Mt. Oryang in Daejeon, South Korea. Since Mt. Oryang is located behind the high school, there are few hiking people and the ecosystem is well preserved. The materials for experiment were attached to trees or put on a stick and positioned on the ground. Then they were attached to trees, the distance between them was various between one and two meters. The distance between the treated group and the control group was more than 5 meters. Several models were hung on a toothpick or a wooden chopstick which was planted in the ground. Numbers were marked on the trees for easy collection of the results of the experiment. Number distanced more than one meter from the model so as not to affect the results of the experiment.

Experiment of avoidance about Peacock butterfly’s eyespots

In Experiment 1, the treated group and the control group were set as follows to investigate if the eyespots of peacock butterfly cause a sense of avoidance for birds (Fig. 1).
- Control group: A single butterfly model without a pattern
- Treated group: A butterfly model without a pattern and another butterfly model with a pattern put together

33 models in the control group and 33 models in the treated group were used respectively for one time of experiment. The control group and the treated group of each set were attached to different trees or planted into the ground in the same place. Different sets of the control group and the treated group were attached to trees and the number of the butterflies without a pattern eaten by birds was counted. At this time, if the butterflies without a pattern in the control group were eaten more, it can be presumed that the butterflies without a pattern in the treated group were eaten less by a sense of avoidance caused for birds by the butterflies with a pattern. However, if there is not much difference in the number of butterflies without a pattern eaten by birds between the two groups, it doesn’t fit the existing theory that the butterflies with a pattern creates a sense of avoidance for birds.

The experiments were conducted three times in total in order to improve the objectivity and secure the reliability of the experiment.
Experiment of avoidance about caterpillar’s eyespots

Experiment 2 was performed in the same way as Experiment 1 using caterpillar model. The control group and the treated group were set as follows (Fig. 2).

- Control group: A single caterpillar model without a pattern
- Treated group: A caterpillar model without a pattern and another caterpillar model with a pattern put together

A total of 50 models were used for the control group and the treated group respectively for one time of experiment. The control group and the treated group of each set were put on different tree boughs or placed on the points with little coming and going and people and animals. At this time, if the caterpillar without a pattern in the control group were eaten more, it can be presumed that the caterpillars without a pattern in the treated group were eaten less by a sense of avoidance caused for birds by the caterpillars with a pattern. However, if there is not much difference in the number of caterpillars without a pattern eaten by birds between the two groups, it doesn’t fit the existing theory that the caterpillars with a pattern creates a sense of avoidance for birds.

The experiments were conducted three times in total in order to improve the objectivity and secure the reliability of the experiment.
Interpretation of results

The results were collected after performance of three repeated experiments in total. The number of observation objects and the number of survived observation objects were written in the table of survival rate by rounding off the arithmetic mean of the results of the 3 times of experiments to the nearest integer. Kaplan-Meier survival analysis was done for the analysis of the results in order to secure a higher precision and objectivity of the experiment. The cumulative survival rate was obtained by using product-limit method for the data of the number of objects collected in the process of performance of the experiment. The cumulative survival rate was shown in the shape of Kaplan-Meier curve for the analysis of the difference in the survival rate between the two groups. Log Rank Test was conducted for examination of the difference in Kaplan-Meier obtained with the data of the control group and the treated group. Preparation of graphs and statistics was done by using GraphPad Prism 8.

Results

Experiment of avoidance about Peacock butterfly’s eyespots

The models used for the experiment were left unattended for 5 hours in total, and the number of remaining objects were counted every hour. Experiment was conducted 3 times and a table and a graph below is rounded off the mean. The table of survival rate was filled in with the counted numbers for calculation of the section survival rate and the cumulative survival rate. Kaplan-Meier curve was drawn on the basis of the cumulative survival rate. For statistical verification, Kaplan-Meier curve went through Log Rank Test to see if there is a significant difference.

Among the 33 observation objects in the control group, 26, 24, 19, 16 and 14 pieces survived in each time slot (Table 1). In the treated group, 31, 27, 24, 23 and 19 pieces survived in each time slot (Table 2) among the 33 observation objects. Cumulative survival rate for 5 hours was 0.424 in the control group and 0.551 in the treated group. Though the cumulative survival rate of the treated group was relatively higher than that of the control group, the section survival rate fluctuated both in all the sections of the control group and the treated group with not much difference in the value itself. Thus, it was difficult to judge that there was significant difference.
Table 1. Survival rate of the control group

<table>
<thead>
<tr>
<th>Observation time (hour)</th>
<th>Number of survived observation objects</th>
<th>Number of observation objects</th>
<th>Section Survival Rate P(t)</th>
<th>Cumulative Survival Rate S(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>33</td>
<td>0.788</td>
<td>0.788</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>26</td>
<td>0.923</td>
<td>0.727</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>24</td>
<td>0.792</td>
<td>0.576</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>19</td>
<td>0.842</td>
<td>0.485</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>16</td>
<td>0.875</td>
<td>0.424</td>
</tr>
</tbody>
</table>

Table 2. Survival Rate of the treated group

<table>
<thead>
<tr>
<th>Observation time (hour)</th>
<th>Number of survived observation objects</th>
<th>Number of observation objects</th>
<th>Section Survival Rate P(t)</th>
<th>Cumulative Survival Rate S(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>33</td>
<td>0.939</td>
<td>0.939</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>31</td>
<td>0.871</td>
<td>0.818</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>27</td>
<td>0.889</td>
<td>0.727</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>24</td>
<td>0.958</td>
<td>0.696</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>24</td>
<td>0.792</td>
<td>0.551</td>
</tr>
</tbody>
</table>

The following is the Kaplan-Meier curve drawn with the given cumulative survival rate (Fig. 3). To compare the two curves with Log Rank Test, Chi square value was 1.497 which is less than the test statistic compared to the chi-squared distribution whose degree of freedom is 1. In addition, the P value was 0.2212 which is more than 0.005 (Fig. 4) showing that there is no statistically significant difference.
Figure 3. Kaplan-Meier survival curve of the control group and the treated group

Figure 4. Chi square and P value of Kaplan-Meier survival curve

*Experiment of avoidance about caterpillar’s eyespots*

The models used for the experiment were left unattended for 5 hours in total, and the number of remaining objects were counted every hour. Experiment was conducted 3 times and a table and a graph below is rounded off the mean. The table of survival rate was filled in with the counted numbers for calculation of the section survival rate and the cumulative survival rate. Kaplan-Meier curve was drawn on the basis of the cumulative survival rate. For statistical verification, Kaplan-Meier curve went through Log Rank Test to see if there is a significant difference.

Among the 50 observation objects in the control group, 45, 40, 38, 32 and 29 pieces survived in each time slot (Table 3). In the treated group, 44, 40, 36, 33 and 29 pieces survived in each time slot (Table 4) among the 50 observation
objects. Cumulative survival rate for 5 hours was 0.58 in both the control group and the treated group and there was not much difference between the two groups.

**Table 3. Survival rate of the control group**

<table>
<thead>
<tr>
<th>Observation time (hour)</th>
<th>Number of survived observation objects</th>
<th>Number of observation objects</th>
<th>Section Survival Rate P(t)</th>
<th>Cumulative Survival Rate S(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>50</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>45</td>
<td>0.889</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>40</td>
<td>0.95</td>
<td>0.76</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>38</td>
<td>0.917</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>32</td>
<td>0.906</td>
<td>0.58</td>
</tr>
</tbody>
</table>

**Table 4. Survival rate of the treated group**

<table>
<thead>
<tr>
<th>Observation time (hour)</th>
<th>Number of survived observation objects</th>
<th>Number of observation objects</th>
<th>Section Survival Rate P(t)</th>
<th>Cumulative Survival Rate S(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>50</td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>44</td>
<td>0.909</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>40</td>
<td>0.9</td>
<td>0.72</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>36</td>
<td>0.917</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>33</td>
<td>0.879</td>
<td>0.58</td>
</tr>
</tbody>
</table>

The following is the Kaplan-Meier curve drawn with the given culminative survival rate (Fig. 5). To compare the two curves with Log Rank Test, Chi square value was 0.06854 which is less than the test statistic compared to the chi-squared distribution whose degree of freedom is 1. In addition, the P value was 0.7935 which is more than 0.005 (Fig. 6) showing that there is no statistically significant difference.
Discussion

Research summary and significance

This study was conducted to present experimental grounds for the role of the eyespots of insects which has been an object of controversy for a long time. In particular, the main purpose of this study is to verify or refute the existing theory that the eyespots of peacock butterfly and caterpillar trigger a sense of avoidance for birds by making them recognize that the peacock butterfly or caterpillar is a predator larger than them. Perceiving that the existing researches lack reliability and cause counterargument by making new models that do not exist in nature, this study aimed to prove the theory by arranging the models in a different way rather than modifying the models of peacock butterfly existing in
nature. In Experiment 1, we prepared peacock butterfly models with eyespots and without eyespots. We set a single butterfly model without a pattern as the control group and a butterfly model without a pattern and another butterfly model with a pattern put together as the treated group. We attached them to trees and counted the number of the survived peacock butterfly models every hour. The results of the experiment were statistically processed by using Kaplan-Meier survival analysis and investigated the difference between the two groups by Log Rank Test. Though the cumulative survival rate of the control group was relatively higher than that of the treated group, there was not much difference in the section survival rate. In Kaplan-Meier curve, the Chi square value was 1.497 and P value was 0.2212 showing no significant statistical difference. It means that it is difficult to see that the eye shape of peacock butterfly triggers a sense of avoidance for birds by making them recognize the eyespots as the eyes of a larger predator. Experiment 2 was conducted in the same way as Experiment 1 on caterpillars. We set a single caterpillar model without eyespots as the control group and a caterpillar model without eyespots and another caterpillar model with eyespots put together as the treated group. We put the models on tree boughs and counted the number of the survived caterpillar models every hour. The results of the experiment were statistically processed by using Kaplan-Meier survival analysis and investigated the difference between the two groups by Log Rank Test. The difference between the control group and the treated group was less than Experiment 1. The Chi square value was 0.06854 and P value was 0.7935 showing no significant statistical difference.

If the eyespots imitated the eyes of a larger predator, a smaller predator would not attack the models feeling a sense of avoidance. Therefore, through Experiment 1 and Experiment 2, it was possible to refute the existing theory that eyespots increase the survival rate by actually imitating the eyes of the natural enemy of the predator.

This study casts doubt about the theory of Edward Poulton which has been accepted naturally over a century without exact verification. So many publications and dissertations have been written based on the wrong existing theories. This study was conducted in order to prevent such an error. The eyespots of insects were an interesting natural phenomenon for numerous scientists. While there are many researches which present various function of the eyespots, few researches have been conducted for verification of the existing research dissertations. The performance of this study will play the role of raising the importance of the verification of the existing theories in various areas of scientific research besides eyespots.

**Consideration**

There are various other opinions on the function of the eyespots reported in many dissertations. Stevens et al. (2008) conducted experiments by changing the pattern of the eyespots into other forms including square and bar shapes. The effect of thwarting predation was not much different in other patterns. Furthermore, they asserted that the color contrast of the eyespots have an important influence on predation and it would be related to the visual disturbance of birds citing that predation was thwarted more effectively when a stronger color contrast was given to the eyespots. Marples and Kelly (1999) and Coppinger(1969) made similar proposals about the color contrast and the function of avoidance of the natural
enemy by the eyespots. About such a claim, Stevens explains that it is because many biological pattern have specific shapes and colors instead of imitating eyes.

In another dissertation of Stevens (2013) and the dissertation of Barber et al. (2003), it was asserted that the sense of hearing is as effective as visual sense for thwarting predation. In fact, predation can be thwarted most effectively if hearing effect is made in addition to eyespots, and the attack of the predator is delayed by maximizing derangement or surprising the predator. It is also reported that changing one’s actions suddenly is effective for thwarting predation and avoiding the attack of the predator.

Jones (1980) examined the degree of the avoiding reaction of birds when they see various kinds of patterns. According to him, while two eyespots made more avoidance than one eyespot, there was no significant difference in the shapes that do not look like eye shape such as two diamond shapes or three circular shapes. He also reports that there was no big difference when the inside of the circle was not filled in. The shape which stimulated avoidance of birds the most was a rectangle. He concluded that the two patterns do not have to be symmetrical circles though the symmetry of the patterns has an influence on thwarting predation.

There are also researches that continue to explain the existence of the eyespots at the level of genes. Nijhout (1980) and French and Brakefield (1992) assert that two symmetrical patterns in circular shape is related to evolution which is distant from imitating the eyes of a larger animal. They say in their dissertations that a circular shape like the eye is the pattern that can be made by genes the most easily and it has little to do with imitating the eyes of a larger predator. They also report that a pair of two eyespots, too, is made by the mechanism of molecular biology by bilateral symmetry of many animals including butterflies and moths.

The existing theories continue to be challenged by diverse researches and approaches that are being made for understanding of eyespots in various ways. The many preceding researches that support the results of this study suggest that new approaches will be made for verification of the existing theories.

This study conducted experiments to verify the oldest hypothesis which explains the role of the eyespots of insects. While this study has effectively shown that the eyespots of insects are not imitating the eyes of a larger predator and predation is not thwarted by creating a sense of avoidance, there is a limitation to selecting and presenting a single theory. Thus, the researches trying to find a new hypothesis about the function of eyespots will continue and the researches for proving it will also have to be continued. It is necessary to conduct researches on processing visual recognition of animals besides human beings and information processing for preparation of more objective proofs. In particular, it is known that birds have more sensitive vision than human beings and thus it is not easy to explain the influence of eyespots without specific research data. We expect that more persuasive hypotheses will be proposed by conducting researches in such a direction on not only animal behaviors but also on other areas including brain science and cognitive science.
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