Investigating *Pieris rapae* co-evolutionary interactions with Brassicas for use as research and teaching models at the University level

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Abstract

We are developing modules incorporating the Cabbage White Butterfly (Pieris rapae) for use in undergraduate biology laboratory courses. P. rapae has co-evolved in both feeding and egg-laying preferences for plants from the family Brassiacea. We are testing it for use as a teaching system to build lab modules that can incorporate the entire life cycle of both the cabbage white butterfly and/or their brassica hosts. This system is flexible enough for students to develop their own testable hypotheses and experiments incorporating large sample size. Student will analyze, discuss, and present their resulting data and conclusions in a scientific paper or other suitable format such as the mini-report contained within our poster. Results of our lab trials using two behavioral modules and a pilot lab on larval feeding preference (General Biology II, Summer 2011) were well received and showed the relative ease of use and utility of the Brassica-Butterfly system.

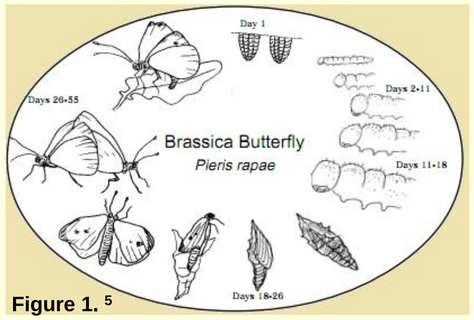

Figure 1. [5]

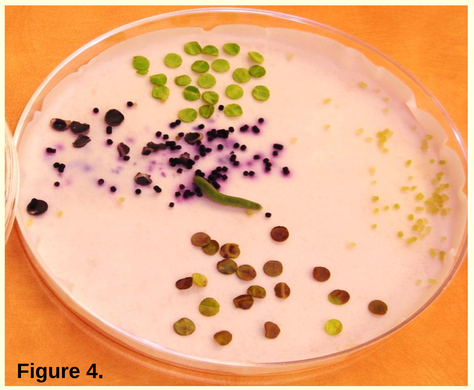

Figure 4.

Figure 5.

Figure 6.

Introduction

The Cabbage White Butterfly **(Fig. 1)** was evaluated as a model organism for college level biology labs (it is already used in AP biology labs[4]). *P. rapae* feed exclusively on Brassicas[2] **(Fig. 2)** , which are all important food crops such as broccoli, cabbages, mustards, bok choy, etc.. *P. rapae* detect[2] the glucosinolates ("mustard oil") found in Brassicas which are absent in non-Brassica plants. These interactions can be the basis of a variety of student inquiries into co-evolution, behavior, and other biological phenomena. Instructors can utilize the Brassica-Butterfly system allowing students to investigate their own hypotheses, design controlled experiments, analyze data, and present their data in any of several formats. We specifically experimented with two modules: larval feeding preference **(Fig. 3 and 4)** and egg laying preferences of female butterflies **(Fig. 5 and 6).**

Caring for Larvae

Butterfly eggs from Carolina Biological Supply were placed on radish sprouts for hatching larvae to eat. As larvae matured, they were fed Brussels sprouts **(Fig. 2).**

Caring for Butterflies

Butterflies were fed 20% honey nectar and kept in a Plant-Light Box available from Carolina Biological. Females laid eggs on provided Brussels sprouts which were used to raise the next generation.

Caterpillar Feeding Module

Students were given a choice of different types of vegetables (including roots, leaves, and stems of Brassica and non-Brassica) and these plants were of many different colors and textures. They could be placed in petri-plate "feeding arenas" for students to test their rationale-based hypotheses dealing with feeding behavior and preferences. Please see the mini-report to the right for an example of our trial feeding preference experiment.

Butterfly Egg-laying Module

We tested two versions of a module based on butterfly egg laying preferences. Females butterflies were left in enclosures with Brassica and non-Brassica ovipositrons (as suggested by Wisconsin FastPlants[5], **Fig. 5 and 6.)** for 48 hours, and provided honey-nectar. Isolated mated females (**Table 2**, "A" and "B") lay eggs only on cabbages (green cabbage (**Fig. 6**) is more favored than red cabbage). In a colony, they also lay some eggs on lettuces. Crowding and aggressive behavior are possible explanations for this observation. We are continuing to investigate experimental design for this module.

Table 2 : Egg-laying preferences for various females (run for 48 hours)

	Green Cabbage	Red Cabbage	Green Leaf Lettuce	Red Leaf Lettuce
Group	240	211	109	13
Individual A	60	11	0	0
Individual B	40	17	0	0

Conclusion:

The Brassica-Butterfly system is flexible for diverse experimental designs and hypothesis testing. Students were curious for further scientific inquiry using this system. Students' interest was apparent in better quality work relative to their non-Brassica-Butterfly related group experiment mini-reports.

Figure 2.

Figure 3.

A Chrysalis

Literature Cited:

1. Chew, Frances S. "Foodplant Preferences of Pieris Caterpillars (Lepidoptera)." Oecologia46.3 (1980): 347-53. JSTOR. Springer in Cooperation with International Association for Ecology. Web. 27 Feb. 2012. <http://www.jstor.org/stable/4216179>.
2. Courtney, Stephen P. "Coevolution of Pierid Butterflies and Their Cruciferous Foodplants IV. Crucifer Apparency and Anthocharis Cardamines (L.) Oviposition." Oecologia 2nd ser. 52: 258-65. Jstor. Springer, 1982. Web. 16 Dec. 2011. <http://www.jstor.org/stable/4216607>.
3. **Harvey, Jeffrey A., Leontien M. A. Witjes, Maria Benkirane, and Roel Wagenaar. "Nutritional Suitability and Ecological Relevance of Arabidopsis Thaliana and Brassica Oleracea as Foodplants for the Cabbage** Butterfly, Pieris Rapae." Plant Ecology 189.1 (2007): 117-26. Jstor. Springer, 2 Sept. 2006. Web. 15 Dec. 2011. <http://www.jstor.org/stable/40212853>.
4. Hoeck, Kathy Van. "Using Brassica Butterflies as a Model Organism for AP Biology Lab 11."*The American Biology Teacher* 72.6 (2010): 361-64. *Jstor.com*. University of California Press on Behalf of the National Association of Biology Teachers, Aug. 2010. Web. 10 Jan. 2012. <http://www.jstor.org/stable/10.1525/abt.2010.72.6.9>.
5. "Investigating Life with the Cabbage White Butterfly and Brasscas in the Classroom- a Model of Organismal Interdependence." 1-40. FastPlants. Wisconsin Fast Plants. Web. 1 Jan. 2012. <Publication no. WFP072402. 1 Sept. 2011. .>.

Example of a Student Mini Report

Which vegetables and in what quantities will *Pieris rapae* larvae consume, when isolated with Brassica and non-Brassica plant leaf samples?

Chuen. J, **Mahon. B.**, Lab Section A: 4/14/12

Introduction The entire genus of Brassica butterfly, *Pieris*, have co-evolutionarily developed an exclusive relationship to feed and depend on Brassica plants.

Pieris rapae are nontoxic but as caterpillars have effective camouflage with most brassica plants—red cabbage may be less appealing for safety reasons, though can be acceptable as food if absolutely necessary (Hovanitz and Chang, 1962). *We thus hypothesize that green cabbage should be preferred over red cabbage and, red or green lettuces (non-Brassicas) by P. rapae.*

Methods

We provided two cabbage varieties (Brassicas)—green and red cabbage– and two organic lettuce varieties (non-Brassicas) as 20 disks each arranged in petri dishes as in **Figure A** for 24 individual caterpillar trials. After 24 hours, disks were assessed for herbivory, and then averages and standard deviations were calculated for each variety. (Chuen and Mahon, in prep).

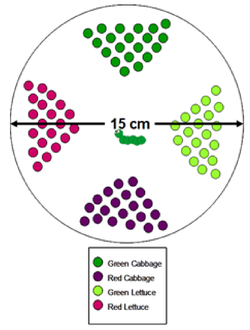

Figure A. Experimental set up for feeding preference.

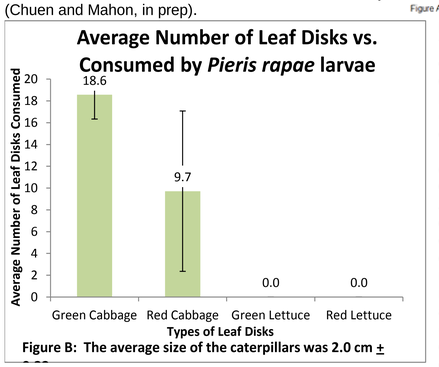

Figure B: The average size of the caterpillars was 2.0 cm ±

Results

The average number of disks eaten by all 21caterpillars (3 did not participate) was much higher for the green cabbage (18.57 ±2.22), than the red cabbage (9.71 ±7.36), green lettuce (0), and red lettuce (0) **(Figure B)**.

Conclusions

Although not significantly different, *Pieris rapae* caterpillars appear to prefer to eat green cabbage more than red cabbage, and did not eat red nor green leaf lettuces. In another parallel experiment (data not shown), larvae completely abstained from eating when only lettuce was provided to them for 24 hours. Our data is in agreement with what has been previously observed by Hovanitz and Chang (1962) that larvae may starve rather than eat an unacceptable, non-host plant. Our experiment failed to distinguish a significant difference between red and green cabbage consumption; future direction may be to have a larger sample supply of plant material to allow preference for one or the other to be demonstrated.

Literature Cited

Chuen, J. and Mahon, B. "Investigating *Pieris rapae* co-evolutionary interactions with Brassicas for use as research and teaching models at the University level" in prep for submission to The American Biology Teacher.

Hovanitz, William, and Vincent C. S. Chang. "The Effect of Various Food Plants on Survival and Growth Rates of Pieris." Journal of Research on the Lepidoptera 1st ser. 1 (1962): 21-42. Lepidoptera Research Institute Foundation. Lepidoptera Research Institute Foundation, 1962. Web. 16 Dec. 2011. http://lepidoptera research foundation.org/journals/01/PDF01/01-021.pdf

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Future Directions: We plan to submit a paper for publication to The American Biology Teacher based on these modules

For further information…

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