Catching butterflies

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This project was to set up a captive breeding programme between *Heliconius hecale melicerta* and *Heliconius hecale zuleika*. Whose wing colour pattern form the basis of two mimicry rings and the project's end aim is to discover the genes that encode them. These two subspecies of butterfly can interbreed, producing fertile offspring, but are found in different geographic locations in Panama. *H. hecale zuleika* are found in western Panama, although their exact range is not known, we collected ours from Bocos del Torros region and *H. hecale melicerta* were collected 'Pipeline Road' in Santa Cruz, in the Panama Canal Zone. Six butterflies were collected from each site and bred to produce virgin offspring of each subspecies who would then be mated to create hybrids. Their wing pattern phenotypes examined and genetic analysis done to establish genetic markers and in the end hopefully discover the genes underlying the resultant colour patterns.

However this report outlines the initial breeding programme: how the butterflies were collected and raised, including the difficulties that were faced at the egg, larval, pupa and adult stages of development.

Methods

The butterflies were collected by netting, with more females captured than males, because they could be caught as they came down to lay eggs on *Passiflora vitifolia*. The butterflies from Bocos del Torros had to be stored in transit in stamp collecting envelopes with a damp rolled up pieces of tissue paper, inside a plastic container, to keep them moist and comfortable, but immobile during the journey. The butterflies were fed twice a day with sugar solution, through a syringe into their proboscis.

Once at the field station the butterflies were kept in large insectaries made of a fine mesh with flowering *Psiguria*, a vine with bright red flowers that acts as a nectar source and *P. vitifolia* as the larvae host plant, for the butterflies to lay their eggs on. Sugar water in plastic containers were also hung from the enclosure to act as an extra nectar supplement. The enclosure had to be kept free of other insects as in the past peaks in mortality have been due to spiders and preying manti! A difficulty we encountered was the presence of ants, who were attracted to the sugar water and may have damaged the eggs and the leafcutters were a threat to our plant supply.

The eggs were collected and housed in plastic containers with a damp paper towel to maintain humidity. When the larvae hatched they were supplied with very young *P. vitifolia* leaves and early on in the project *Passiflora biflora* leaves also, as the larvae grew they were fed older leaves. When the larvae had reached their fifth instar, they were moved into either Ziploc bags or larger plastic containers so that they could pupate. The resulting butterflies would then be moved outside into the insectaries.

However we faced a high mortality at the larval stage, so we set up an experiment to examine the larval feeding plants. It is known that they eat *P. vitifolia* and for practical purposes due to its scarceness and slow growth rate, it was hypothesised that they could be fed the close relative *P. biflora* too. So we set up 20 feeding purely on *P. vitifolia,* 20 on *P. biflora* and 20 with a choice of either.

Results

We had a sample size of twenty larvae in each group, the day to day data can be found in the appendix. From Figure 1, it is clear that the best survival rates are seen in larvae fed on only a *P. vitifolia* diet, with 85% survival. Those fed on just *P. biflora* had only 5% surviving and it was observed that the majority of them died over the 1st two days after hatching. However the larvae that were able to choose their diet were quite successful, with an end rate of 50% survival.

Figure 1: Population size at stages during development

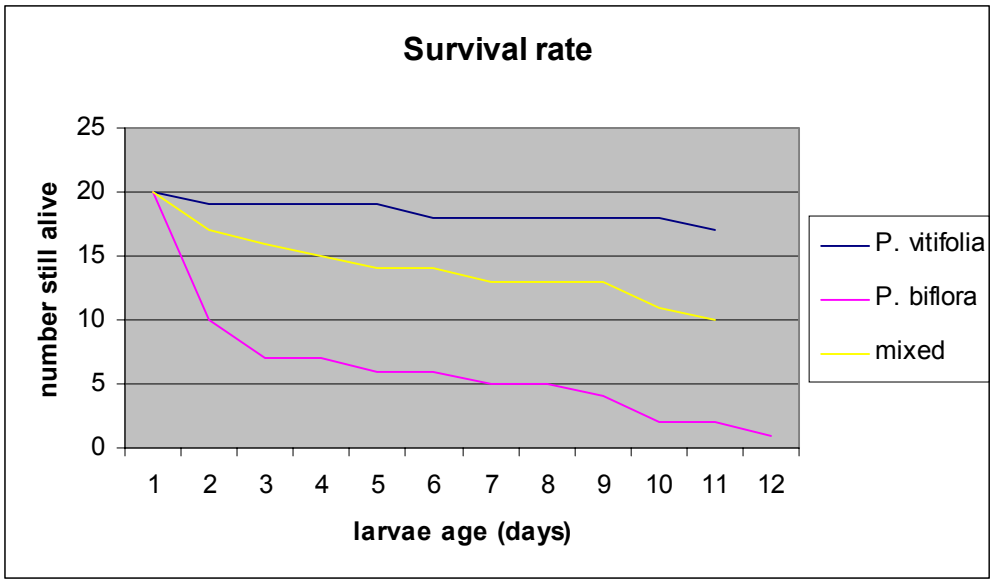

Table 2: Mean length of population over time

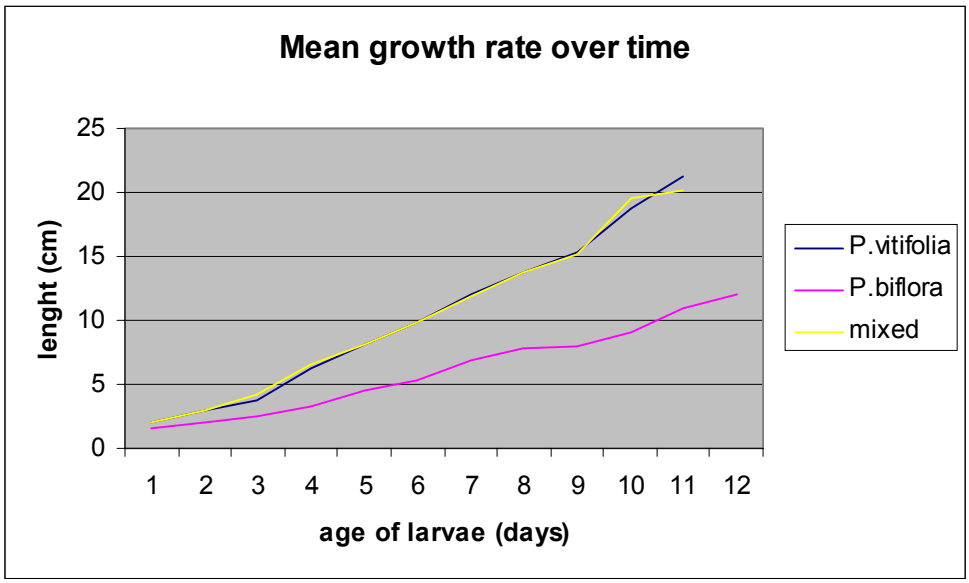

Figure 2 examines larvae growth rate, calculated by the mean length of the surviving larvae at that day of development. The results of Figure 1 have to be taken into

account as although each category starts out with 20 larvae, this sample size decreases as some of them die. However it does show that those that eat the purely *P. vitifolia* diet grow at the same rate as those on the mixed diet, but the ones eating just *P. biflora*, not only die young, but those that survive have a much slower growth rate. It is possible that the ones on the mixed diet thrived so well because most of the time they chose to eat the *P. vitifolia* and therefore it is understandable that they would show the same patterns of growth and the deaths could be accounted for when they had to eat *P. biflora* as had run out of *P. vitifolia.*

From the information gathered and highlighted on the graphs, it is apparent that both *H. hecale melicerta* and *H. hecale zuleika* can thrive on a *P. vitifolia* diet but that the consumption of *P. biflora* is leading to stunted growth and death!

Discussion

This summer's work has shown that it is possible to raise *H. hecale melicerta* and *H. hecale zuleika* in captivity, but we did have a number of problems. The experiment demonstrated that the larvae are selective feeders only eating *Passiflora vitifolia*. We also found that the young larvae required younger leaves, this has been noted by Young (1975) in the wild too, as the young are found on terminal and subterminal leaflets. We observed that they liked to be on their own, as when a number were housed together you would see them waving their upper bodies at eachother.

We encountered difficulties at the pupal stage too: problems with high levels of humidity in the Ziplocs, resulting in occasional fungi growth. Also the pupae had difficulty attaching to the plastic, so we added plant material and they attached to that and were less likely to fall off. Another problem with the Ziplocs was that they were too thin, there was not enough room for the butterflies to stretch their wings when they first immerged, which resulted in one poor crumpled butterfly. Due to this we changed to larger plastic tubs.

Once the difficulties were overcome we ended up with a number of *H. hecale melicerta* and *H. hecale zuleika* which happily bred together to create the F1 generation which is now growing n Panama.

This project was essential for developing methods to raise the different stages of the butterflies, without which, it would not be possible to continue with the genetic wing pattern studies. There is also the possibility that this information could be used in the butterfly farming industry, which is flourishing in Panama presently with increased tourist demand to see the wonderful butterflies living here.

Acknowledgements

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Bibliography

Picture on front cover from **www.elbosquenuevo.org/ butterflies/pictures/**

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Summer Time Plan

I undertook this project with the Smithsonian Tropical Research Centre for 6 weeks, here is a rough rundown of how that time was spent.

Week 1: sewing enclosures
Capturing *Heliconius hecale melicerta*
Test breeding some larvae, 100% mortality due to humidity and diet
Collecting *Passiflora vitifolia* to make lots of cuttings

Week 2: trip to Bocas del Torros, collecting adult *Heliconius hecale zuleika*
Searching for *H. hecale zuleika* eggs and larvae

Week 3: self breeding *Heliconius hecale zuleika* and *Heliconius* self-breeding *Heliconius hecale melicerta* to establish virgin offspring of each species
Collecting resulting eggs and larvae, trying to rear

Week 4: success with larval stage
Collecting more plant samples and locating further sources as larvae consumption is huge.

Week 5: larvae reaching pupal stage
Problems with Ziplocks, experimenting with different ways of containing them

Week 6: first butterflies emerging!

Finances

Incoming

Barnson Bequest	£300
British Association Travel Fund	£500
James Rennie Bequest	£400
Wier Fund for Field Studies	£1000
Total	£2200

Outgoing (approximately)

Flights Flights - return Glasgow to Panama	£769
Fieldwork travel and accomidation	£100
Transport to work	£50
Travel insurance	£122
Accomidation	£320
Medication	£250
(vaccinations, 1st aid kit, malaria pills)	
Equipment:	£370
(mosquito spray, appropriate clothing, film, torches, etc)	
Subsistence	£200
Total	£2171