

Instructions for participants:

Measuring latitudinal changes in herbivore predation rates using artificial caterpillars

Introduction

Insect herbivores and insect herbivory are generally thought to increase towards the tropics (Andrew, Roberts & Hill 2012). Interactions between organisms are also thought to be stronger in the tropical regions (e.g. Coley & Barone 1996; Pennings & Silliman 2005) (but see Moles *et al.* 2011 for review of evidence); however, whether the predation of insect herbivores by higher trophic levels decreases with latitude has never been studied. To investigate how predation rates differ with latitude we have therefore designed a global citizen science experiment. Using a standardised study on a global scale, and the concerted effort of a large number of ecologists, will allow us to assess quantitatively how general and consistent such patterns are.

Questions

Using this global approach, we will address the following questions:

1. Does predation of insect herbivores decrease with latitude?
2. Which are the main predators of insect herbivores?
3. Do the predators of insect herbivores change with latitude?

Rationale

To estimate relative predation rates and the importance of arthropods, birds and small mammals as predators of a major category of herbivores (lepidopteran larvae), one may use artificial plasticine caterpillars (Loiselle & Farji-Brener 2002; Richards & Coley 2007; Tvardikova & Novotny 2012). While plasticine caterpillars lack some of the characteristics of real caterpillars, they have been shown to suffer similar levels of attack, and so give a comparable measure of predation between predators and habitats (Richards & Coley 2007; Howe, Lövei & Nachman 2009). This method has been successfully used to indicate predation rates in both tropical (Loiselle & Farji-Brener 2002; Koh & Menge 2006; Posa, Sodhi & Koh 2007; Richards & Coley 2007; Faveri, Vasconcelos & Dirzo 2008; Howe, Lövei & Nachman 2009; Tvardikova & Novotny 2012) and temperate (Skoczylas, Muth & Niesenbaum 2007; Lluch *et al.* 2009) ecosystems.

Site locations

To maximise the power to detect latitudinal gradients, we are aiming for a good spread of sites across latitudes and land masses. Our primary criterion for site selection is "naturalness". The site you use should be subject to minimal disturbance; a national park or other protected area is desirable.

Ideally, the site should be in a habitat as close as possible to the climax vegetation type. For example, if forested habitats are the climax vegetation type, the site should not be situated in grassland.

We are not restricting our sampling to sites of a particular vegetation type, because we consider the fact that vegetation type changes with latitude to be an ecologically important global pattern, which is likely to influence global patterns in herbivory, herbivore densities and predation. Thus, we aim to establish sites in the most abundant natural vegetation type in each area.

Exact site locations will be determined according to:

1. site condition (avoid close proximity to roads, vegetation edges, and sites of major disturbance),
2. ease of access/contact in the area,
3. low risk of public interference.

Equipment

100 plasticine caterpillars in tubes + 10 spares

100 labels for tubes

Flagging tape (orange)

Waterproof marker

2 tubes of superglue

Safety pins to open glue

Datasheets on waterproof paper

Hand lens (x10 magnification)

Pencil

Return address prepaid envelope (if applicable in your country)

Protocol

To provide a standardised bait, we have moulded artificial caterpillars (2.5 x 30mm) for you from odourless, non-toxic coloured plasticine (Lewis Newplast in an equal mixture of two colours: green and light green) to resemble undefended, green geometrid larvae. This type of caterpillar was chosen as it represents the most abundant group of Lepidoptera found throughout the world. The caterpillars have been moulded into the characteristic looping position of a geometrid caterpillar (commonly adopted whilst resting). The caterpillars may have become slightly stuck to the inside of the tubes while in transport. If this is the case they can usually be dislodged by banging the tube firmly on a hard surface or tapping the side to knock them out. If possible avoid putting anything inside the tube, as this may mark the caterpillar. Once out of the tube inspect the caterpillar for any marks. Small marks (such as marks from fingernails) can usually be

smoothed over, or replace badly damaged caterpillars with the spares provided.

It is critically important that we employ standardised methods to ensure that sampling is done in a consistent way across the sites, so please follow these instructions carefully.

Caterpillars should be placed on naturally-growing seedlings, or shrubs not more than 1m above the ground. To capture differences in predation rates, even where these are low, we need the sampling effort to be high. We have therefore supplied you with 100 caterpillars. These should be distributed among five 3m x 4m grids with 20 caterpillars in each grid. Each caterpillar within a grid should be separated by at least 1m, and individual grids should be a minimum of 50m apart (see Figure 1). Ideally, all grids should be set up on the same day, but if this is not possible, grids can be set up on different days. Grids do not need to be exact and one large step can be used to measure 1m. You can use the flagging tape provided to aid location of the plants, but place the tape at least 25cm from the caterpillars to avoid the flag affecting predator activity. Tie the flagging at the bottom of the vegetation near the ground and always to the right of the plant with the caterpillar on. Placing the marker in a consistent way will help you re-find the caterpillars. It would be helpful if you could take at least one photograph of each grid, so we have a record of the habitat.

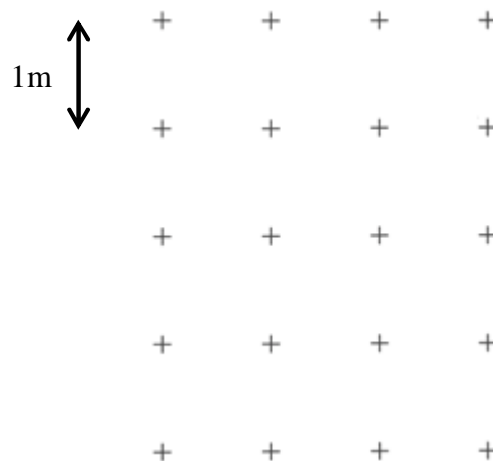


Figure 1. Layout of one grid of 20 caterpillars (+ = caterpillar).

1. As we cannot not control for plant species used throughout the study, we ask that you place the caterpillars on the upper side of leaves that are undamaged and of a similar size and shape (i.e. undamaged, simple, entire leaves). Make sure that the leaves are not damaged or touching the ground as this can affect predator behaviour and access.

Number the underside of the leaves sequentially before you attach the caterpillars with the non-toxic pen provided. Number the flagging tape with the same number to help you keep track of the caterpillars when you collect them in. The numbering of the leaves is important because you need to be certain that you have checked every single leaf that a caterpillar was attached to: sometimes the caterpillar may have fallen off and the leaf is only recognisable from its number.

2. Use the glue provided, and place a small drop on each end of the caterpillar and fix tightly to the upper side of the leaf along the midrib. If the leaf is flexible, placing the caterpillar close to the stem will help it stay in place. Make sure that the leaf is dry and clean before applying the glue and hold in place for about 1 minute until the glue sets. If the air is very dry then breathing on the glue will add the necessary moisture for hardening. Check the caterpillar for any imperfections that could be mistaken later for predator damage, and be careful when attaching the caterpillar to avoid making marks (e.g. from finger nails). Care should be taken to minimise the amount of glue used as this may give unwanted chemical signals to predators. We have provided 10 extra caterpillars, so caterpillars damaged in transit can be replaced.
3. As we predict that attack rates will vary, we ask that you check the caterpillars three times: after 24h, 48h and 96h (4 days) \pm 2 hours from the time they were first placed in the field. This will allow us to calculate time until attack as well as overall predation rates. If you would like to continue checking the caterpillars for a longer time period (particularly if attack rates are low) then please remember to record the number of days of exposure for each caterpillar when you collect it in.
4. When checking the caterpillars, use the datasheet provided to record the data for each caterpillar. You should here indicate the date when the caterpillar was collected in, whether each caterpillar has been attacked or not, by which predator group (see below), and the number of days of exposure. Use the hand lens and guide supplied to determine whether the individual shows signs of being attacked (see Figure 2). If lighting conditions are poor, a head lamp may help you to see the marks more clearly. Collect each caterpillar that shows any signs of attack (and leave the rest of the caterpillars in place). Take extra care when removing the caterpillar from the leaf to avoid leaving marks (e.g. from fingernails) in the plasticine. Once you have inspected the caterpillar presumed to be

damaged, then place it in the tube provided and label the tube with the following information:

Place, Date collected in, Number of the grid, Number of the leaf, Number of days of exposure, Your initials.

5. If you decide after inspecting the caterpillar that it does not show any signs of attack, glue it back on to the leaf.
6. Each predator group can be distinguished due to characteristic attack marks (See 'Attack marks guide', Figure 2 below and photos on the website). Arthropods, such as ants, make small slits and scrape marks from mandibles, birds leave characteristic beak marks, and mammal attacks can be recognised from incisor marks and scrapings (rodents), or rows of pointed teeth marks in the case of shrews. Classify the attack as "unknown" if it is ambiguous. Snails, slugs and woodlice sometimes eat the plasticine. They tend to eat the whole caterpillar, leaving only 'crumbs' or leave rasping marks (See Figure 2). As this is not a true predation event, we will not include these in our analyses. These should therefore be marked as S=Snail/Slug on the datasheet and a note made in the comments box about the damage. Fill in the sheet with A=arthropod, B=bird, M=mammal, or U=unknown. If the caterpillar shows signs of being attacked by more than one predator, the letters of all possible predators should be recorded. It doesn't matter how many attack marks are on the caterpillar from each predator – we are only recording presence or absence of each predator type.
7. If the caterpillar has fallen off the leaf, but can be retrieved then either glue it back onto the leaf if it shows no sign of attack, or if it has been attacked then indicate any attack marks on the sheet, and tick the box "fallen" indicating that it had fallen off the leaf. If the caterpillar cannot be found, please tick the box "lost" indicating this.
8. After you have completed the experiment please post back all the caterpillars in their labelled tubes and the datasheets in the prepaid addressed envelope. Please email the photographs of the grid to sfegtweets@gmail.com, or share via Dropbox / Google Drive / similar. If you have taken any other photographs you think would be of interest to us (e.g. of attacked caterpillars) then please do also send these. You may keep the other bits of equipment, please don't mail them back to us as postage costs can be high.

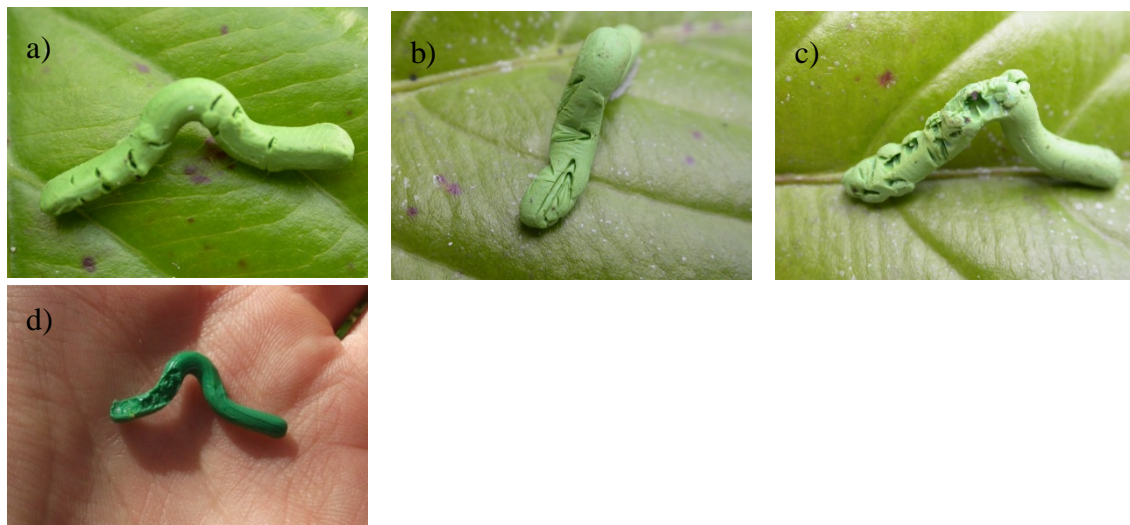


Figure 2. Characteristic attack marks of ants (a), birds (b), rodents (c), and slugs/snails (d).

Any questions?

IF YOU ARE UNSURE ABOUT ANYTHING, PLEASE ASK!!!

Remember that it is CRUCIAL that the sampling is done in a consistent way across the sites. We would much rather have you ask for advice in tricky situations than decide on your own. We will be checking e-mail as regularly as possible and will do our best to get back to you quickly.

e-mail us at: bess.hardwick@helsinki.fi

We aim to publish a global paper that will synthesize the overall results from all the sites. We will invite all contributors to participate in the writing of this paper, and will offer you the opportunity to be authors on the resulting publication.

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