How to avoid being eaten!

An investigation into the resting posture of peppered moth caterpillars (*Biston betularia*) during the day

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An ASAB teaching resource for GCSE, A Level and Advanced Higher Biology students and for AS/A2 Psychology students

Background notes for teachers

Moths, at all stages of their development, are preyed upon by a variety of predators: for example, ants eat eggs, wasps eat larvae, mice eat pupae and birds eat adults. Although some predators, like mice and bats, are active at night most moth predators are hunting during the day. So the behaviour of moths during daylight is critical to their survival.

Moth larvae use a number of different strategies to enhance their survival during the day. A number of species are cryptic and rely for survival on keeping still and blending in with the background on which they are resting. The caterpillars of some species masquerade as objects in the enivironment, such as twigs and bird droppings. Some larvae hide in the soil or under leaves to escape attacks by ground and aerial predators. The larvae of a number of moth species have a dense covering of hairs, which are believed to make swallowing difficult. The caterpillars of some species rest in the open amongst the vegetation, masquerading as twigs, but are well-camouflaged and keep their bodies rigid and still. One species employing these strategies is the peppered moth (see Figure 1), whose caterpillars feed and rest on a variety of plants, such as birch, hawthorn, oak, elm, lime, beech, plum and other fruit trees, and ground flora like bramble and rose. The larvae hold their bodies rigid and motionless throughout the day, unless they are disturbed by another animal walking along the twig or branch they are resting on, or by a prod from the beak of a predatory bird.

Initial observation suggests that the angle of rest of peppered moth caterpillars on their chosen twigs may be relatively constant so this investigation provides an opportunity for students to determine if this is the case. Peppered moth larvae are fairly short-lived, emerging from the egg in June or July and pupating in late September. So it is probably advantageous if the neural circuitry of a larva already has the necessary instructions for taking up an appropriate daytime resting posture.

Figure 1 Typical daytime resting posture of a peppered moth larva

Larval colouring

Caterpillars have to be some colour. Many of them, such as larvae of the small white butterfly, are green and live among green foliage. Observation and experiment shows that green caterpillars are usually edible, leading to the conclusion that greenness provides protection when against green backgrounds. Some other edible moth and butterfly larvae are dark brown, but are difficult to see against the dark twigs on which they stand while feeding on the attached leaves. This is true of many peppered moth caterpillars, but others are a paler olive colour, a fact noted as long ago as the 18th century by Moses Harris, see Figure 2. The related scalloped hazel moth exhibits both these colour forms and in some places a form with a lichen pattern. These moths feed on numerous species of plants, which themselves differ in colour, so that it is advantageous to have the right match on each plant. But how do the larvae achieve the match?

Figure 2 Green and brown peppered moth caterpillars

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In some species colour is genetically fixed. In many cases, however, a direct response of the insect to its environment determines its appearance. Caterpillars of one swallowtail butterfly resemble bird droppings at first, but late larvae have a pattern which camouflages them among the leaves of host plants. This change is controlled by hormones and can be reversed experimentally by injecting the hormone present in the young caterpillar into more advanced individuals. Experiments with other swallowtails show that the colour of the pupal case (the chrysalis) is changed depending on what the caterpillar can see; if the surroundings are green the pupae are green but otherwise they are brown. Larvae of some tropical moths vary in colour and in the number of spiky dorsal projections, depending on the type of host plant occupied. In some swallowtail butterflies the larvae have one colour and pattern when they feed on a narrow-leaved plant, regardless of what kind of plant it is, and a different one when they feed only on trees or shrubs. In all these cases, a stimulus received through the eye probably affects hormone production which, in turn, causes the particular colour or pattern.

Temperature is another factor than can influence larval and pupal colour. Larvae of species as different as monarch butterflies and cabbage moths are darker when reared at low temperature (12˚C) than high (24˚C). Finally, larvae of some moth species are affected by density, so that caterpillars reared in isolation tend to be green/brown and difficult to see while those reared in groups are black and highly visible. Larvae transferred from isolated to crowded conditions, or from crowded to uncrowded, immediately change their coloration, which again suggests that being the right colour is important. Each spring, when moths go through their larval stage, many small birds, such as blue tits, hunt for them intensively to feed their young, so there is strong pressure to evolve protective adaptations.

Instead of camouflage, another protective adaptation is to become inedible. Some caterpillars do so by feeding on poisonous plants and retaining the poisons in their bodies. When that happens they are often brightly coloured and hairy. The hairs are irritants, producing strong allergic reactions when they come into contact with skin. They are therefore a direct deterrent and bright colours act as a warning signal, see Figure 3. But nothing is ever perfect, and some predators are adapted to specialize on prey other predators avoid. One such is the cuckoo, which is often abundant near colonies of scarlet tiger moth and cinnabar moth caterpillars, both distasteful to most birds and conspicuously black and yellow. If more predators are put off than become specialists, however, such adaptations will be retained over evolutionary time.

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Figure 3 Scarlet tiger moth (top) and cinnabar moth caterpillars (bottom)

The Peppered moth has been the focus of quite extensive research, especially during the twentieth century, and so we know much about their ecology and behaviour.

Ecology and behaviour of peppered moths and their larvae

The peppered moth, *Biston betularia*, is a nocturnal species but needs to rest safely during the day out of the view of their main predators – birds. Their principal strategy is to camouflage themselves, and their white wings with black speckles provide a good match against lichen covered bark upon which they rest, see Figure 4.

Figure 4 Peppered moth resting on an apple tree [The moth has been placed here in order to take the photograph – adults normally rest on branches higher up in the tree canopy.]

During the industrial revolution of the nineteenth century this lichen flora was killed by pollution and the bark blackened with soot. With the typical form now exposed to predators on the blackened tree branches, another form of the peppered moth began to increase in frequency – the melanic form *carbonaria*, which had a selective advantage of being more camouflaged on the blackened bark, see Figure 5.

Figure 5 Melanic and typical forms of the peppered moth

It was for this reason that the peppered moth went on to become one of the most quoted examples of natural selection, made famous by Bernard Kettlewell's classic mark-release-recapture experiments in the 1950s. He demonstrated that birds selectively predate upon the moths which were exposed, showing how the melanic form was able to increase in number. Later experiments showed that the moths also chose backgrounds that matched the colour of their wings i.e. the melanics preferred black backgrounds, and the typicals preferred white backgrounds. The mechanism behind background selection was researched in a number of species in the 1960s and 70s, and for the peppered moth it was concluded that it is genetic, and probably controlled by the gene (s) for wing coloration.

The peppered moth lives in its adult form for only a few weeks, and in that time must reproduce to pass on its genes to the next generation. The adults emerge from their pupae from March up until late August. Males crawl up a tree or shrub (or any nearby vertical surface) and wait for their wings to expand and dry out before flying off in search of females. In the peppered moth, the females are generally sedentary and do not move far from where they emerge, instead hanging with an organ extended that produces pheromones detected by the large feathery antennae on the males. Neither sex feeds, instead they exist off fat stored in the body from when they were larvae.

The moths are nocturnal and mating takes place at night, with the adults remaining together for up to 24 hours. The female then lays her eggs within fissures or under the bark of the tree she is on, taking up to a week to do so, see Figure 6. She lays up to several hundred eggs which normally hatch within a couple of weeks of being laid. Like an army marching into battle, once free of their eggs the larvae set off up the tree and out towards the tips of branches, where they suspend themselves from a thread of silk and wait to be blown away by the wind. This is a dispersal technique, similar to that used by spiderlings, and the larvae could be carried several kilometres from their natal tree.

Figure 6 Eggs and pupa of the peppered moth

Unlike many other species of butterflies and moths, the larvae eat a wide range of food plants – very useful if it is uncertain where you may end up! In fact peppered moth larvae have been recorded on hundreds of different species of food plant across the northern hemisphere, including oaks, willows, birches, fruit trees, roses, hawthorn and brambles. When they hatch, the larvae are only about 2 mm in length, and dark in colour with pale stripes on the sides of the body. They usually have 5 moults, with each stage between the moults (instar) spent feeding almost continuously and growing into each new skin. The larvae stay on the leaves of the food plant for the first couple of weeks, and as they get bigger begin to rest on the twigs of the food plant.

To avoid being eaten, as they grow they gradually begin to resemble twigs, with bud-like heads and knobbles on the body - the mimicry being enhanced by their resting position on the twig. The larvae are able to mimic the colour of the twigs on which they rest – on dark twigs such as birch, the larvae are dark brown in colour, and on bright green twigs such as willow the larvae are green, see Figure 2. Even more extraordinarily, the larvae are also able to change from one colour to another, although this is a slow process unlike the colour changes seen in chameleons (which is controlled by the nervous system) and appears to need moulting to take place for a complete colour change. It appears to be visually induced, with the food ingested only playing a small role.

The larvae are fully grown in about 2 months, and are up to 6 cm in length and as thick as a pencil. Hormones in their bodies that controlled moulting and growth now signal the larva to stop growing and to pupate. They head downwards from the food plant, and once they reach the ground they burrow themselves into soil, usually going down a few centimetres. Once there they have one final moult, and with their skin comes all the features of their larval body which they will no longer need (including the jaws and prolegs – the fleshy grasping 'legs' on the abdomen of the larvae). The cuticle (outer skin) hardens and the insect within begins metamorphosis, where larval organs are reabsorbed into the body, and adult features (such as wings) being to develop. As the environment cools, the metabolic processes within the pupae slow to a stop and the pupae go into diapause over the long winter months. This stage is the longest in the life cycle of the peppered moth, and once the spring weather comes, the pupae come out of diapause and finish metamorphosis. The fully formed adults emerge and the cycle begins again.

With this background, we outline here a suitable investigation for students into the resting posture of peppered moth larvae during daylight. After a brief introduction we provide a suitable line of enquiry for GCSE, AS/A2 Level, Advanced Higher students of Biology and AS/A2 Psychology students. We also provide a short Powerpoint presentation which teachers may find helpful in setting the scene for the investigation.

Investigation

Introduction

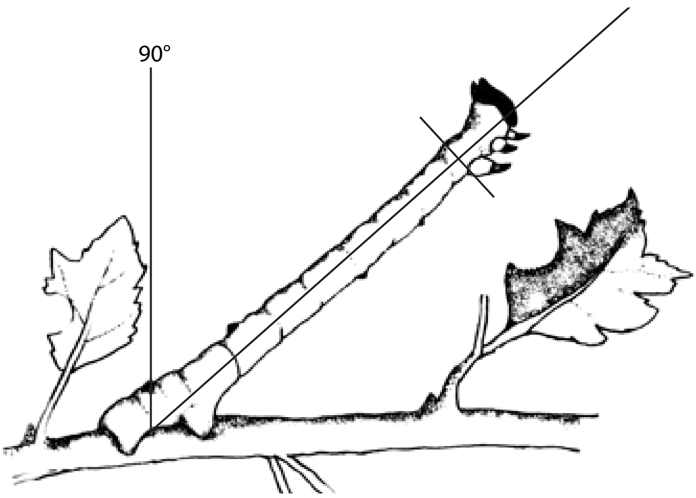
The aim of the investigation is to determine if, during the hours of daylight, caterpillars of the peppered moth show a preferred angle of rest, i.e. is the angle relatively constant? Students will look at a series of images of larvae and measure the angle of rest of each one. They will then assess, using calculations and graphs, and perhaps a statistical test, whether larvae do show a preferred angle of rest. We finally hint at why this behaviour may have evolved.

For GCSE students we have provided photographs of 15 different peppered moth larvae as the stimulus material: for A Level and Advanced Higher students there are 24 images. [Obviously, teachers can adjust these sample sizes as they wish.] The images used in the investigations were selected at random from a series of images taken over a period of 3 weeks in August 2008. Each image was taken with the same digital camera (a Nikon Coolpix S9) by either MD or JM and the image stored. All the larvae photographed were reared on hawthorn, *Crataegus monogyna*, throughout this 3 week period until all the photographs had been taken.

To take the image, each larva, resting on a twig, was placed on top of a standard school protractor, with the twig aligned to run along the horizontal line (0˚) on the protractor. Students are required to read off the angle of rest (it is easier to always read the acute angle) between the larva and the twig.

A larva`s body obviously has width so we suggest the following method is used which will, hopefully, produce a consistent method of measurement for the angle of rest. Firstly, mark, with a dot, where the 90˚ vertical line on the protractor meets the body of the caterpillar on the twig. Secondly, draw a line across the body of the caterpillar just behind the final pair of legs. [The line is usually 3 – 8 mm long.] Mark, with a dot, the centre of this line and then join the two dots together, extending the line so that it crosses the semi-circular edge of the protractor. This enables the student to read off the angle of rest of the larva to the hawthorn twig, see Figure 7, and an example is illustrated in Figure 8.

Figure 7 Drawing to illustrate the technique for determining the angle of rest of a larva



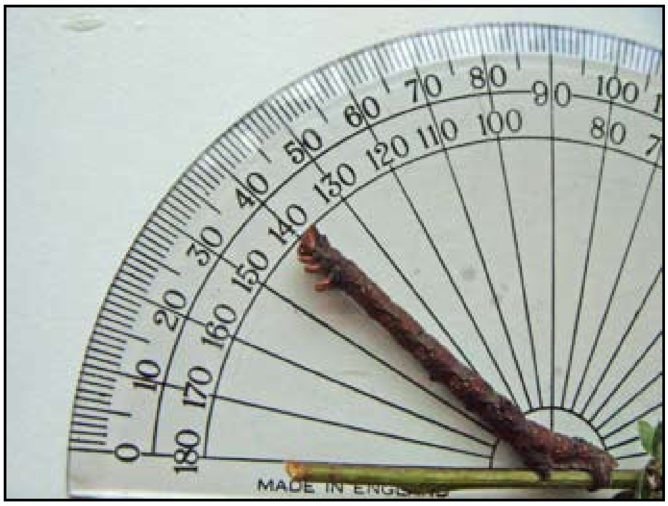


Figure 8 A caterpillar resting at an angle of 41˚ to a hawthorn twig

We have provided examples in the two files, N = 15 for GCSE and N = 24 for A level and Advanced Higher students, which should ensure that the line that is drawn between the two dots does not pass outside the body of the larva. If this technique is followed it will provide a consistent method for measuring the angle of rest of caterpillars. [We suggest that teachers allow 1 or 2˚ leeway for the angle of rest measurements, which we have listed in the `suggested` answers section.] Students can record the angles on one of the check sheets provided, see Tables 1 and 3.

Teachers may wish to use some, or all, of the suggestions below, or they may prefer to devise their own questions. [A full set of data are in Table 1 in the appendix and Table 2 lists the photo codes (and letters) and twig angles for the GCSE and A Level exercises.]

GCSE BIOLOGY

[For students following the Edexcel GCSE Biology course, this investigation and the follow-up exercises would be especially relevant for teaching sections B3 2.1, B3 2.16 and B3 2.18. The suggestions here could also be used by students following other exam board specifications, as an exercise in data representation and analysis, for example.]

1. Measure the angle of rest (to the nearest degree) of each of the peppered moth larvae and record the angle on Table 1 for the fifteen caterpillars, A – O.

2. Using these data, complete Table 2 to show the frequency of occurrence of the angles of rest in three angle of rest classes: 0 – 29˚, 30 – 59˚ and 60 – 89˚. Draw a suitable graph to represent these data.

3. Calculate the mean, median and mode values of the angles of rest for the sample of fifteen caterpillars.

4. Referring to your graph, suggest ONE reason why you think the graph has this pattern.

5. Suggest TWO factors that you could investigate if you were to carry out a follow-up study of the resting position of peppered moth caterpillars during daylight.

Teachers may wish to use this investigation to illustrate aspects of scientific enquiry. Here are just a few suggestions.

- Why it is important that the same technique is used to construct the line for the measurement of the angles?
- What alternatives techniques could be used?
- What is the scale of measurement that is used to determine the size of the angles?
- What is the accuracy of the measurement of the angles?
- What procedure is to be used for lines that pass midway between two angle divisions on the protractor? Do you round up or round down?
- What problems may arise in measuring angles from photographs?
- What variation occurs within a class, or small group of students, in the measurement of the same angle of rest?
- What are the potential sources of bias in using photographs for this work?
- Was using two photographers a good idea? Would they each use the camera in exactly the same way?
- Why might it be useful to have `trial runs` at reading larval angles of rest from photographs before embarking on the `real study`?
- What confounding variables may be influential?
- Was the sample size reasonable, too large (or time consuming) or too small?
- What are the disadvantages of using secondary data sources?
- Would replicating this investigation in your own school be advantageous?

Table 1 Angles of rest of peppered moth larvae

Caterpillar	Angle of rest (°)
A	
B	
C	
D	
E	
F	
G	
H	
I	
J	
K	
L	
M	
N	
O	

Table 2 Frequency of occurrence of the angles of rest of larvae in 3 classes

Angle of rest	0 - 29°	30 - 59°	60 - 89°
Frequency			

[For students following AS/A2 and Advanced Higher Biology courses and International Baccalaureate Biology syllabuses, this investigation and the follow-up exercises would be especially relevant for any of the sections dealing with stimulus and response, innate and learned behaviour and their influences on survival, natural selection, anti-predator strategies, etc.. For courses with minimal behaviour elements (this may apply to AS/A2 Psychology courses too), the suggested exercises could prove useful in teaching aspects of research techniques, such as hypothesis testing, use of statistical tests, data representation and analysis, etc..]

The same procedure can be used for the measurement of the angle of rest of larvae to the hawthorn twig, as described above. We have provided 24 images of larvae (A – X) and a second check sheet, see Table 3.

1. Measure the angle of rest (to the nearest degree) of each of the peppered moth larvae and record the angle on Table 3 for each of the twenty four caterpillars, A – X.

2. Using these data, complete Table 4 to show the frequency of occurrence of the angles of rest in five angle of rest classes: 0 – 17˚, 18 – 35˚, 36 – 53˚, 54 – 71˚ and 72 – 89˚. Draw a suitable graph to represent these data and then comment on the pattern.

3. i) Calculate the mean and standard deviation of the angles of rest for the peppered moth caterpillars A – X.

   ii) Explain what the standard deviation of a set of values is.

   iii) Why is it important to know the mean and standard deviation of a set of values?

4. One suggestion why caterpillars may show a preferred angle of rest is that the angle of their bodies to the twigs is similar to the angles of any side shoots or twigs that are found on stems of hawthorn, see Figure 9.

   Selecting 24 separate stems on a hawthorn tree or bush (or birch or other tree that is easily available and is a food plant of the larvae) in your school grounds, measure the angle at which ONE twig diverges from the hawthorn stem using a school protractor, see Figure 10. Try to choose the stems you measure in an unbiased way. Record all the angles in the appropriate column in Table 3.

   [N. B. Students need to be aware that hawthorn twigs have several very sharp thorns along their stem which can easily pierce the skin of the unwary. They need to take care when carrying out this task, even under the necessary supervision of teaching staff. They should also wash their hands after completion of the measurements. If cuts and scratches occur they should be immediately dealt with in accordance with the relevant health and safety guidelines of the particular school or college.]

5. With the two sets of angles listed, draw a suitable diagram or graph to show the data you have collected, both the angles of rest of caterpillars and the angle of divergence of hawthorn stems. You may find that a dispersion diagram will neatly represent these data. Once the individual values are identified in each of the two columns of the dispersion diagram, calculate the median, upper quartile and lower quartiles for each set of values and show these 3 values in each column, see Table 1 and Figure 1 in the Appendix for the data for twig angles of hawthorn trees in Urmston. Comment on the distribution of the two sets of values.

6. In addition, or as an alternative to question 5, use a statistical test to determine if there is a significant difference in the angles of rest of peppered moth caterpillars and the angles of divergence of hawthorn stems. Make sure you use an appropriate statistical test and record all the information you have from your statistical testing, especially if you use a computer package to carry out the analysis.

7. Peppered moth larvae feed on many plants, such as rose, bramble, willow, birch and elm, as well as hawthorn. Repeat the angles of divergence measurements for ONE other food plant in your school grounds. Are your results similar or different to those collected when measuring hawthorn twigs?

Figure 9 Caterpillar on a typical hawthorn twig with side shoots

Table 3 Angles of rest of peppered moth larvae and angles of divergence of twigs on hawthorn (or other species)

Caterpillar	Angle of rest (°)	Angle of divergence (°)
A		
B		
C		
D		
E		
F		
G		
H		
I		
J		
K		
L		
M		
N		
O		
P		
Q		
R		
S		
T		
U		
V		
W		
X		

Table 4 Frequency of occurrence of the angles of rest of larvae in 5 classes

Angle of rest	0 - 17°	18 - 35°	36 - 53°	54 - 71°	72 - 89°
Frequency					

Evolutionary aspects of the behaviour

Figure 10 a and b Student using a protractor to measure the angle of a twig to a hawthorn stem

Both ©Dave Maskew

Findings

The resting angles of peppered moth larvae, at least on hawthorn, are not constant, as the standard deviation values confirm. So peppered moth caterpillars vary in their angle of posture when resting on a hawthorn twig (or other species) during the day. This is advantageous since their dispersal method as larvae means that the wind could blow them away to land on a variety of tree species, with twigs at varying angles.

Why has the behaviour evolved?

A peppered moth larva relies on camouflage and keeping its body rigid and motionless to increase the chances of survival during daylight when birds and other predators are actively hunting. Holding its body rigid and static is more likely to convince a predator that it is a twig. If the angle of rest of its body to the hawthorn stem it is resting on mimics the angles of a `real` hawthorn twig (or that of another plant), then this posture serves to enhance survivability. However, the actual angle does not appear to be as critical as rigidity and keeping still.

This deceit will be even more convincing to predators if the larvae are the colour of hawthorn twigs (or those of other plants). Peppered moth caterpillars are generally either green, with some brownish blotches, or brown, see Figure 2. Thus, larvae with these colours and larvae which hold their bodies at similar angles to plant stems as `real` twigs, will be afforded the greatest protection since they will be more convincing as twigs. Larvae which were of different colours (i.e. not green or brown), or larvae which moved around and engaged in feeding during the day, would be selected against since they would be more readily detected and eaten by predators.

`Suggested` answers

Powerpoint - slide 3

Left – be well camouflaged and masquerade, as a twig, (a peppered moth caterpillar)

Right - be hairy (a garden tiger caterpillar)

Middle top – hide under a leaf (a common quaker caterpillar)

Middle bottom – be poisonous (a cinnabar caterpillar)

GCSE Biology

1. A (photo code 3250) = 33˚, B (3364) = 20˚, C (3308) = 30˚, D (3369) = 53˚,

E (3475) = 71˚, F (3479) = 45˚, G (3473) = 47˚, H (3543) = 36˚, I (3551) = 40˚,

J (3522) = 30˚, K (3575) = 40˚, L (3644) = 50˚, M (3683) = 32˚, N (3886) = 47˚ and

O (3335) = 38˚

2. 0 – 29˚ = 1, 30 – 59˚ = 13, 60 – 89˚ = 1 [an alternative would be 15 – 29˚ = 1, 30 - 44˚ = 8, 45 – 59˚ = 5, 60 – 74˚ = 1]

3. mean = 40.80˚, median = 40˚, mode = 30˚, 40˚ and 47˚ [stand. devn. = 12.18˚]

4. the histogram shows a distribution that is not a classic normal distribution for the 3 angle of rest classes, even though all three central tendency measures are within the same modal class – a normal distribution is frequently found for biological data provided the sample size is reasonable, but this one may be rather small so the fit isn`t close

5. does the angle of rest vary on different tree species? – are the angles of rest on trees with several tree species in the same genus (such as willow) similar? – are individual caterpillars consistent in their angle of rest? – do caterpillars show similar angles of rest if they are provided with artificial twigs, like plastic?

A Level and Advanced Higher courses

1. A (photo code 3250) = 33˚, B (3364) = 20˚, C (3308) = 30˚, D (0790) = 55˚,

E (3463) = 46˚, F (3417) = 50˚, G (3475) = 71˚, H (3484) = 38˚, I (3473) = 47˚,

J (3551) = 40˚, K (3554) = 31˚, L (3575) = 40˚, M (3571) = 34˚, N (3553) = 45˚,

O (3644) = 50˚, P (3642) = 42˚, Q (3825) = 48˚, R (3683) = 32˚, S (3886) = 47˚,

T (3885) = 32˚, U (3335) = 38˚, V (3485) = 32˚, W (3338) = 41˚ and X (3341) = 32˚

2. 0 – 17˚ = 0, 18 – 35˚ = 9, 36 – 53˚ = 13, 54 – 71˚ = 2, 72 – 89˚ = 0 – the histogram shows a distribution that is not a classic normal distribution for the 5 angle of rest classes even though all three central tendency measures are within the modal class (36 – 53˚), – a normal distribution is frequently found for biological data provided the sample size is reasonable and the fit to a classic normal distribution might be expected to be better with an increase in sample size

3. i) mean = 40.58˚, standard deviation = 10.50˚ [median = 40.0˚ and mode = 32˚]

ii) the standard deviation of a set of scores/values is a measure of the dispersion of individual scores around the mean

iii) the mean and standard deviation describe the nature/shape of the distribution of a set of scores/values – the two values allow researchers to determine if the distribution of a set of scores/values accords to a normal distribution, as this is important when deciding which statistical test to use to analyse the data

4. [see student data]

5. [see student graph or diagram – a dispersion diagram for a set of hawthorn twigs measured in Urmston is in the appendix (N = 28): for these data, the lower quartile is 54˚, the median is 60˚ and the upper quartile is 66˚] [see student comments comparing the two distributions]

6. [see student answers]

[For the data presented here, i.e. the angle of rest of 24 peppered moth caterpillars and the angle of divergence of 28 hawthorn shoots from their twig, a suitable test would be a Mann Whitney test: this produces a W value of 1006.0 and an associated probability level of $P = 0.000$, so there is a significant difference between the angles of rest of caterpillars and the angle of divergence of hawthorn shoots from twigs. [A t-test, for unpaired data or independent samples, could also be used and this produces a value of $t = 6.34$, $df = 49$, $P < 0.001$, so again there is a significant difference between the angles of rest of peppered moth caterpillars and the angle of divergence of hawthorn shoots from a twig.]

What does this mean? The fact that caterpillars and hawthorn shoots/twigs are not statistically similar may relate to the fact that peppered moth caterpillars feed on a variety of food plants, and the angles of twigs/shoots on each species probably differ, so selection pressure may be on caterpillars to be of the correct colour and remain motionless and rigid on their chosen twig during the hours of daylight, rather than holding their bodies at a particular angle to a twig - the angle that peppered moth larvae are to the plant stem may not be too critical, since birds are probably not searching for caterpillars that are at a particular angle to a twig

7. [see student answers]

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Useful websites would be:

http://www.nhm.ac.uk/research-curation/research/projects/host-plants/

http://www.ukmoths.force9.co.uk/ - this is the website of UK Moths

Acknowledgements

We are very grateful to Roy Leverton, Reg Fry and Ian Kimber (UK Moths website) for allowing us to use some of their images in this resource and to Judy Evans for her illustration of a peppered moth larva on a hawthorn twig. We also thank Dave Maskew for taking the images of the student. MD and JM took all the other photographs and NE, and the School of Biological Sciences in the University of Liverpool, kindly supplied the larvae. Particular thanks must go to sixth form students Phoebe Mottram (Grange School, Hartford, Northwich) and Hannah Titley (Altrincham Grammar School for Girls`) for volunteering to trial the A Level and Advanced Higher exercise during their summer work experience in 2008 with MD at Manchester Metropolitan University and to the Headmistress, Catherine Russell (Biology teacher) and members of her Year 10 GCSE Biology group at Altrincham Grammar School for Girls` for trialling the GCSE exercise. We are especially grateful to Mick Hoult (MMU) for designing the resource.

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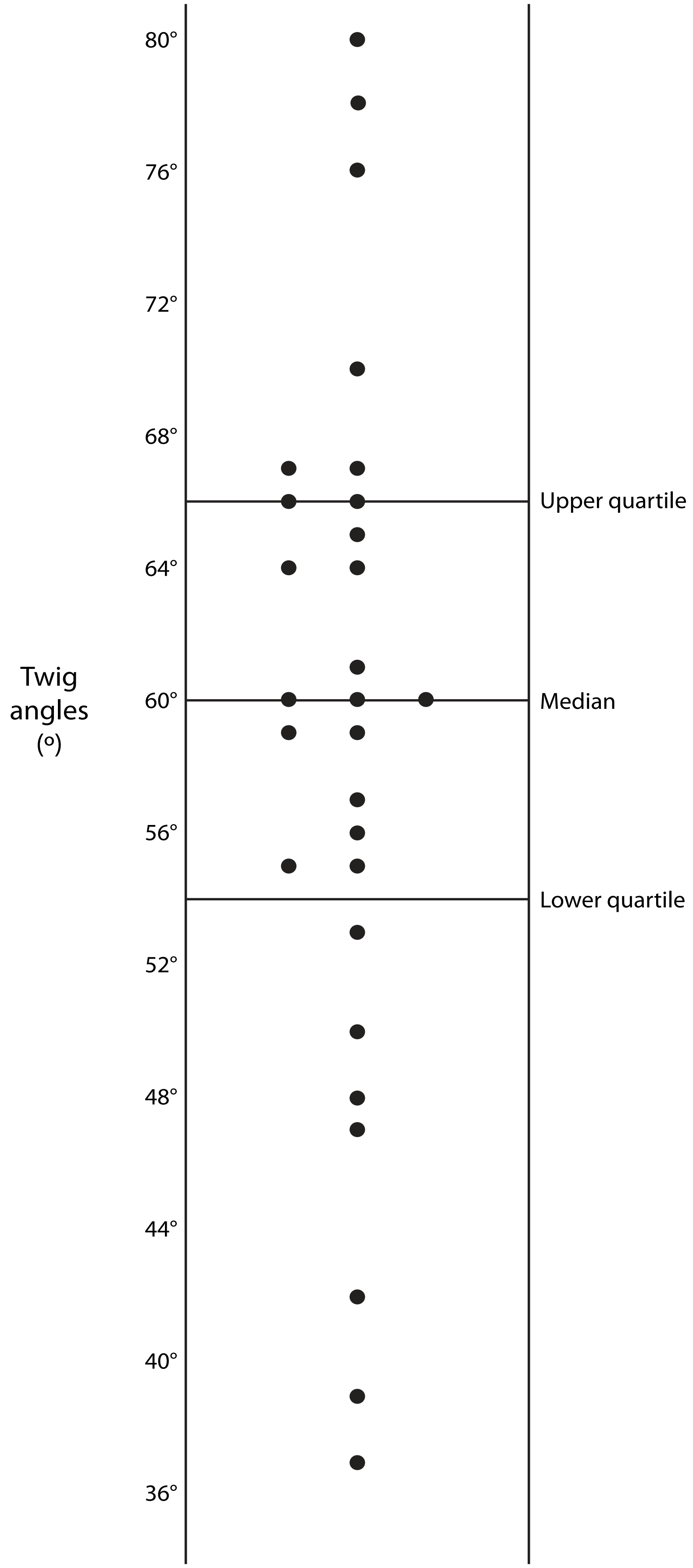
Appendix

Figure 1 Dispersion diagram for the twig angles of hawthorn trees in Urmston



Appendix

Table 1 Data set for hawthorn twig angles and larval resting angles for all moths and for the GCSE samples and AS/A2 samples

Twigs/Larvae	Hawthorn twig angles (Urm)	Larval angles	Photo code	GCSE	AS/A2
1	78	33	3250	33	33
2	59	20	3364	20	20
3	80	30	3308	30	30
4	76	55	790	53	55
5	70	46	3463	71	46
6	64	50	3417	45	50
7	48	53	3369	47	71
8	47	71	3475	36	38
9	59	38	3484	40	47
10	64	45	3479	30	40
11	60	47	3473	40	31
12	61	36	3543	50	40
13	67	40	3551	32	34
14	42	30	3522	47	45
15	53	31	3554	38	50
16	50	40	3575		42
17	66	34	3571		48
18	65	45	3553		32
19	55	50	3644		47
20	60	45	3646		32
21	37	42	3642		38
22	39	48	3825		32
23	66	32	3683		41
24	67	47	3886		32
25	57	40	3923		
26	56	32	3885		
27	60	38	3335		
28	55	32	3485		
29		41	3338		
30		45	3468		
31		32	3341		
32					
33					

Table 2 Twig angles and photo codes (and letters) for the GCSE and A Level exercises

Twigs	Larval angles	Photo code	GCSE	Letter (code)	AS/A2	Letter (code)
1	33	3250	33	A - 3250	33	A - 3250
2	20	3364	20	B - 3364	20	B - 3364
3	30	3308	30	C - 3308	30	C - 3308
4	55	790	53	D - 3369	55	D - 0790
5	46	3463	71	E - 3475	46	E - 3463
6	50	3417	45	F - 3479	50	F - 3417
7	53	3369	47	G - 3473	71	G - 3475
8	71	3475	36	H - 3543	38	H - 3484
9	38	3484	40	I - 3551	47	I - 3473
10	45	3479	30	J - 3522	40	J - 3551
11	47	3473	40	K - 3575	31	K - 3554
12	36	3543	50	L - 3644	40	L - 3575
13	40	3551	32	M - 3683	34	M - 3571
14	30	3522	47	N - 3886	45	N - 3553
15	31	3554	38	O - 3335	50	O - 3644
16	40	3575			42	P - 3642
17	34	3571			48	Q - 3825
18	45	3553			32	R - 3683
19	50	3644			47	S - 3886
20	45	3646			32	T - 3885
21	42	3642			38	U - 3335
22	48	3825			32	V - 3485
23	32	3683			41	W - 3338
24	47	3886			32	X - 3341
25	40	3923				
26	32	3885				
27	38	3335				
28	32	3485				
29	41	3338				
30	45	3468				
31	32	3341				
32						
33						
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