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I'm a worm, get me out of here

Natural selection of spaghetti 'worms'

Aim

This protocol provides an open-ended investigation of directional selection, using wild birds to select 'worms' made from spaghetti.

Introduction

A hundred and fifty years ago, Charles Darwin published his explanation of why there are so many different living things on Earth, each of them so well adapted to its environment. Others had tried to explain this observation before but unlike them, Darwin had spent years collecting evidence to support his theory of evolution by natural selection.

Many people think that Darwin's explanation of how life has evolved (and continues to evolve) is the most important idea ever to occur to a scientist. Even so, it is really a very simple idea at heart — so simple in fact, that one of his friends, Thomas Henry Huxley, said, after reading *On the Origin of Species*:

"... how exceedingly stupid not to have thought of that".

Darwin's principal idea to explain evolution was natural selection. He realised that:

living things produce offspring that vary — they are not all the same;

there is a shortage of resources (food, light, places to live, animals to mate with *etc.*);

individuals that do best in this 'struggle for existence' will tend to leave more offspring, passing on their successful features to subsequent generations.

Therefore, over time, organisms with certain characteristics will become more common, while others will become less common and may die out. Over a long period of time, these small differences between individuals add up to large differences, and eventually new species that are no longer able to interbreed are produced. The practical investigation described here provides a simulation of natural selection, using birds to help in the process: it's bird-powered evolution.

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Bird-powered evolution

The method described here is based on a general technique developed by John Allen and his colleagues and students between the late 1960s and early 1990s to simulate several aspects of evolution by natural selection [1, 2]. The procedure uses different-coloured baits ('worms') that are selected and eaten by birds. Uneaten 'worms' are counted after predation and the 'worm' population is replenished in proportion to those colours which remain. After several cycles of predation and 'breeding', the proportions of the colours in the population change, simulating directional selection. This technique was at one time well-known and widely used in British schools, where it formed a useful practical exercise to accompany the classic example of natural selection by predation that is often cited in textbooks, that of the peppered moth, *Biston betularia* [3].

This practical procedure has numerous advantages over other approaches to the teaching of evolutionary principles, such as computer simulations. These benefits include the active involvement of the students in planning the experimental design and analysing the data, the use of real organisms in the selection process, low cost and ease of preparation. The work is equally well-suited to schools in urban and rural areas, and provides opportunities for sharing data between schools or classes.

The exercise demonstrates selection, a key principle of Darwinian evolution, and could, through a consideration of avian vision and bird colouration, help to introduce students to Darwin's other great evolutionary principle, sexual selection [4].

IMPORTANT NOTE

Critical to the success of this investigation is the selection of a suitable area in which to feed the birds. Ideally, this should be close to a window (for easy observation) and near a hedge or trees so that birds can head for cover (many species do not like to be in the open for long). For birds to feed freely, the area must not be disturbed during the day, either by humans or predators such as domestic cats. If you find that the 'worms' disappear at night, the most likely cause is nocturnal animals such as foxes, cats or rats. If either of these factors is a problem, a solution may be to set up a bird table that is inaccessible to predators, and possibly overseen by a webcam so that the area can be observed without disturbing the birds.

Outline of the practical activity

After acclimatising the local bird population to a new source of food, equal numbers of two different-coloured spaghetti 'worms' are presented to birds. Each day (or at regular intervals), the remaining 'worms' are allowed to

'breed' and the two colour morphs are replaced in proportion to those which remain. Over time, the proportion of the type of 'worms' that are left uneaten by the birds increases, thereby simulating directional selection.

Students should be actively involved in planning the experiment, setting it up, and collecting and analysing the data. It may also be necessary for them to carry out some initial investigations to determine which types of bait will be taken by the local bird population and the best location for the bird feeding area.

This work can be carried out by small groups of students but because the number of potential bird feeding sites will be limited it is probably best undertaken as a whole class investigation.



Equipment and materials

Needed by each class

Dried spaghetti, 500 g

Food colouring, red, 40 mL

Food colouring, black, 40 mL

Food colouring, green, 2 x 40 mL

5 mL pastettes, 3 (for dispensing food colouring)

Wild bird food, 500 g

Sealable plastic bags, 50 (for storing 'worms')

Disposable polythene gloves, 25 pairs (for handling 'worms', especially when they are to be removed from the feeding area)

Biodegradable twine, ~10 m (for marking out feeding area)

Golf tees, 20 (for marking out feeding area)

Bird identification book or chart

Rulers, 150–300 mm (for measuring the 'worms')

Scissors or relatively blunt knives *e.g.*, plastic picnic knives (for cutting spaghetti)

Hand-washing facilities and paper towels

If students prepare their own 'worms'

Bunsen burners, tripods and gauzes, and heat-proof bench mats

OR

Hotplates

Large glass beakers or small saucepans

Eye protection

OPTIONAL

For setting up and observing/recording the investigation

Bird table

Webcam and motion-triggered software for capturing images of birds taking 'worms' *e.g.*, Macintosh: *Evocam* (www.evological.com/evocam.html); Windows: *webcamXP* (www.webcamxp.com)

Digital camera for photographing the feeding area

Binoculars

Advance preparation

Unless the area used for the work is one in which birds already feed, it is essential to 'teach' the birds that food is available there. We suggest that food (such as seeds *etc.* that are sold for feeding wild birds) is placed in the feeding area every day for *at least a week*, and preferably *two weeks* before starting the experiment. You may wish to supplement this food with bread or other food scraps to attract a wider range of species to the feeding area.

The investigation itself takes place over several lessons; it requires students to collect data between lessons and it also presents opportunities for preparatory and follow-up tasks (for example, as homework).

Lesson 1: Planning the investigation

Students will need to be introduced to the principle of natural selection and be given a general outline of the practical investigation and its purpose. The students should then, as much as possible, be involved in planning the precise details of the investigation. Teachers may wish to allocate 'worm' production, data recording and other tasks to individuals or groups of students. It will also be necessary to organise maintenance of the 'worm' selection (bird feeding) area, including the replacement of the 'worms'.

According to the ability and motivation of the group, the planning exercise might involve all or some of the following:

selection of suitable 'worm' colours;

selection of suitable background colours;

location of the bird feeding area;

deciding how the worms are to be distributed within the feeding area, and how many are to be placed there;

deciding on the length of the 'worms' (both to prevent birds selecting prey by size and also so that half-eaten 'worms' can be identified);

the frequency of data recording and when records are to be taken;

the method of counting 'worms' and replacing ones that have been eaten;

the procedure for recording half-eaten or pecked 'worms';

the procedure for counting 'worms' that have been thrown out of the feeding area;

how to account for periods when counting is not possible, such as weekends;

the procedure for observing bird feeding (if possible);

the procedure for recording the results;

how the results will be presented and analysed.

Prey and background colouration

Most of the investigations of this type reported in educational journals aim to study the effect of cryptic colouration rather than directional selection. Consequently the prey are usually replaced to maintain a constant 1:1 ratio of the two colours. In one investigation of crypsis, Allen, Anderson and Tucker [7] used wooden trays filled with painted stones and gravel as a background upon which pastry prey were placed. The authors noted that it was very laborious to produce these trays of stones and to match the colour of the prey to the backgrounds. Cooper, Raymond and Allen [6] later used plain and striped painted boards as a more convenient background. But as noted above, these attempts to match prey and background may have been ineffective due to differences between bird and human vision. Several studies have also noted that certain species may select particular colours of prey regardless of the background colour [1, 2].

In the current investigation, the aim is to simulate directional selection, so the background and prey colour is important only in so far as that there should be a difference between the rates at which the two types of prey are selected by birds. Colour, relative brightness, shape, size, smell and taste of the prey could all influence selection as well as factors such as prey movement (or lack of it). There is some recent evidence to suggest that the double cone structures in birds' eyes may help them to detect movement [8].

It will not be possible for students to allow for all of the factors that could influence prey selection. It is important from an educational point of view, however, that students recognise and attempt to control some of these factors. Students could prepare coloured or patterned boards on which to place the worms for example. Most people would not wish to go to this trouble however. An alternative is simply to use the ground (*e.g.*, grass or

bare soil) as Allen *et al* did in their original experiments, but if this is done it is essential to mark the area clearly so that it can later be searched for remaining 'worms'. A peg or golf tee at each corner with twine between them is sufficient to mark out the feeding area and ensure that the 'worms' are easily located for counting.

Allen *et al* reported that the birds in their studies generally preferred brown over green-coloured baits. They did not suggest a reason for this preference. To humans, green 'worms' are camouflaged against a grassy background, but birds see over a wider range of the electromagnetic spectrum. Green 'worms' may fluoresce brightly in ultraviolet light, making them easy for birds to spot. Students could research information about bird colour vision and choose the colours of their test 'worms' and the background on which they are to be displayed accordingly. They may wish to test whether particular colours fluoresce in UV light. Of course this only gives a very crude indication of how the 'worms' might appear to a bird — but it helps to emphasise to students the importance of such factors when designing the experiment.

If appropriate, students may wish to carry out an initial trial to help them select 'worm' and background colours for the experiment (for instance, our own tests show that pigeons tend to avoid bright red 'worms', whereas more than 50% of green 'worms' were eaten each day).

Test area

As various investigators have reported, the test area can be relatively small, even bird-table-sized *e.g.*, 500 mm x 500 mm [6] or 410 mm x 410 mm [7]. In our trials we marked out two areas of grass: one 1 m x 1 m and another 2 m x 2 m. The larger plot was chosen so that numerous birds could feed in the area at the same time (a flock of large pigeons visited the area daily). In practice, it took a long time to collect and count the worms from this larger plot. This may not be a problem if a small group of students does the counting, but it is probably more convenient to use smaller, 1 m x 1 m, plots or an even smaller bird table.

Location

The test area can either be located on the ground or on a bird table (the latter would prevent predation). Locating the test area close to areas of human or predator activity may of course deter birds from feeding there. Ideally, the feeding area should be close to a window (for easy observation) and near a hedge or trees to provide cover for the birds (many species do not like to be in the open for long). The feeding area may be located where it can be observed by a webcam.

Prey density

To reduce the effect of sampling error that may occur with smaller numbers of 'worms', a high stocking density is preferred. In one investigation, Allen, Anderson and Tucker [7] placed 40 prey on 410 mm x 410 mm trays (20 'worms' of each colour), that is, a prey density of 238 per square metre, which they admit is far greater than would normally be encountered for cryptic prey. We suggest that the area and prey density are chosen to assist with data collection and later calculations by students (*e.g.*, a round number of square metres and prey numbers that can readily be converted to percentages). Because the level of predation will vary, it is not possible to suggest a prey density that would be appropriate in all situations. We suggest that prey are scattered at random at a density of between 50 and 250 per square metre, depending upon the level of predation encountered and the frequency of data collection.

Lesson 2: Preparing the spaghetti 'worms'

Spaghetti is a convenient, robust and inexpensive form of bait. The teacher should explain how the coloured pasta is prepared, cut into worm-sized lengths and stored. If appropriate, the students may cook and colour the spaghetti themselves. (It may be possible to 'borrow' a food preparation area with cookers and hotplates to do this rather than using Bunsen burners, tripods and beakers in a science lab.)

Alternatively, the pasta can be cooked and coloured in advance by a teacher or technician, then students can cut it into worm-sized lengths. The spaghetti must be dyed at least two different colours as it is cooked, then cut into identical lengths (50 mm is ideal). If time permits, students may place the first lot of worms in the feeding area during this lesson. Alternatively, the 'worms' may be stored in sealed containers or plastic bags either in a fridge (for no more than a few days) or frozen until required. (If the spaghetti strands stick together during storage, they can be loosened with a little water.)

The simplest and quickest method of colouring spaghetti is to add the food dye to the water in which the dried pasta is to be cooked (without adding oil to the cooking water). After simmering the pasta for 10–12 minutes, the coloured water can be disposed of. Although a second or third batch of pasta could be cooked in the same water, we do not recommend this as there is a progressive reduction in the intensity of the colour of the pasta which may lead to difficulties in reproducing the experiment should this prove necessary.

Pasta coloured in this way can be handled without excessive amounts of dye transferring to the hands. The dye binds to the pasta and does not readily wash out, even during heavy rainfall.

Individuals will no doubt wish to try different colours and colour intensities but for guidance we found that a grass-like green colour can be obtained by adding 15 mL of green colouring to a litre of cooking water. Similarly, a dark red/brown colour can be achieved using a mixture of 5 mL of black colouring and 10 mL of red colouring in a litre of water.

Should individual students or groups wish to experiment with their own colours these quantities can be reduced for convenience in the school laboratory *e.g.*, using 250 mL of coloured water in 500 mL beakers.

Concentrated food colouring will stain skin, so those handling it may wish to wear protective gloves. If bench surfaces etc. are stained by the concentrated colouring, it can usually be removed using dilute bleach.

Sufficient 'worms' have to be prepared so that they can be replaced each time they are counted (see below). Students will need to understand how to calculate the numbers of worms that should be placed in the feeding area each day, and this can be explained during this lesson.

Equipment and materials

Required by each student or working group

Dried spaghetti (20 strands are sufficient for 100 x 50 mm 'worms'). Note: 'Quick cook' spaghetti may not take up the food colouring before it becomes overcooked and therefore difficult to handle.

Food colouring: 5 mL of black, 10 mL of red and 15 mL of green were required to make brown and green 'worms' as described above.

5 mL pastettes, for dispensing the food colouring (one for each type of food colouring)

Bunsen burner, tripod and gauze or hotplate

500 mL glass beakers or small saucepans, 2

Glass rod, spoon or similar for stirring the spaghetti while it is cooking

Safety glasses (if appropriate)

Pair of scissors or blunt knife (such as a plastic picnic knife) for cutting the spaghetti into worm-length pieces

Ruler for measuring the 'worms'

Plastic bags or sealable containers for storing the 'worms'

Additional equipment required by the class

Large plastic sieve or colander for straining the cooked 'worms'

A clock to time the cooking

Heat-proof gloves for handling the beaker of water

Access to a fridge or freezer for storing the 'worms'

Waterproof marker pen for labelling the bags of 'worms'

OPTIONAL: Chilli powder to add to the 'worms' to deter predation by mammals

Procedure

If you have not been given ready-cooked and coloured spaghetti to cut up, use the 5 mL pastettes to accurately dispense a known amount of food colouring into a measured volume of water (about 15 mL of food colouring is generally required per litre of water). Hint: when you use the pastettes, it is easier to measure volumes accurately if you press the part of the bulb where it joins the stem of the pastette. Your teacher may suggest the amounts of colouring to use to obtain particular colours.

Put on safety glasses.

Bring the water to the boil.

Add the spaghetti. As it softens, use a glass rod or a spoon to push the spaghetti strands under the water.

Turn down the heat so that the water just simmers gently. Cook the spaghetti for no more than 10–12 minutes, stirring occasionally to prevent the spaghetti from sticking to the bottom of the beaker or saucepan. If you overcook the spaghetti, it will be soft and therefore difficult to handle without breaking; if you don't cook it for long enough, it will not take up sufficient colouring. It is important to time the cooking to get the worms 'just right'.

When the spaghetti has cooked, turn off the heat and carefully pour away the water, collecting the spaghetti in a sieve.

Use a ruler and pair of scissors or a knife to cut the spaghetti strands into equal lengths (each about 50 mm long). Take care not to stretch the 'worms' as you cut them. Unless you have decided otherwise, they should all be the same length.

Store the spaghetti 'worms' in a labelled plastic bag to prevent them from drying out until they are needed. The 'worms' can be stored in a fridge for a few days or frozen for a longer period if you wish. If the 'worms' stick together during storage, they can be unstuck using a little water.

Safety guidelines

Commercial food dyes, such as those available from supermarkets are safe for students to handle. The *Royal Society for the Protection of Birds* (RSPB) in the UK has advised that it is acceptable to feed pasta to birds.

Where necessary, students should wear eye protection when heating liquids which, of course, should always be properly supervised.

Between lessons, daily Counting and replacing the 'worms'

It will be necessary to organise a rota for students to count and replace the worms each day, in pairs or small groups. Students will probably have to do this in their lunch break or at another convenient time.

The easiest way to count the worms is to collect all of the remaining worms and to subtract this number from the number of worms that were put out on the last occasion. Appropriate numbers of fresh 'worms' should then be placed in the feeding area. *Note that it is therefore essential that the students understand how to calculate the replacement ratios.* Between lessons: Placing, counting and replacing the 'worms'

There is one important disadvantage to using spaghetti rather than the pastry baits preferred by Allen et al. After 24 hours (or less, in sunny and/or windy conditions) spaghetti will dry out. In wet conditions, the 'worms' will begin to disintegrate after 48 hours. This means that the 'worms' have to be completely replaced every time they are counted (probably every day). This can be an advantage, as counting then merely consists of collecting all the remaining spaghetti 'worms' and subtracting their number from the number that were placed originally. This helps to overcome one of the practical problems identified by Allen, namely that 'worms' can be missed or counted repeatedly. (Allen suggested using a strip of wood or cane that was gradually moved over the test area and that prey were counted when the strip crossed over them.)

Equipment and materials

Required by each student or working group

Golf tees and twine to mark out feeding area, or a bird table

Metre rule or tape measure

Two varieties of prepared worms *e.g.*, two different colours

Containers in which to collect the worms *e.g.*, jugs or plastic bags

Disposable plastic gloves

Soap, water and paper towels for hand-washing

OPTIONAL

Webcam and motion-triggered software for capturing

images of birds taking 'worms' *e.g.*, Macintosh: *Evocam* (www.evological.com/evocam.html); Windows: *webcamXP* (www.webcamxp.com)

Digital camera for photographing the feeding area

Binoculars

Bird identification book or chart

Procedure

A. Placing the 'worms'

Measure out a feeding area in which to place the 'worms', marking it, if necessary, with golf tees and twine (if you use a bird table, you won't need to do this).

Place equal numbers of each of the types of 'worms' in the feeding area. The 'worms' will probably be scattered at random.

OPTIONAL: Take a photograph of the area to record the positions and numbers of the 'worms'. Ensure that you identify the photograph *e.g.*, by date. *Note that this will only be practical for small feeding areas, such as a bird table.*

OPTIONAL: Train a webcam on the area and set up the motion-detecting software so that photos are taken only when movement is detected in the feeding area, enabling you to identify which species of bird (or other animals) have visited the feeding area.

B. Counting and replacing the 'worms'

OPTIONAL: Photograph the feeding area and use the photo for counting the numbers of each type of 'worm' remaining. This will only be practical for small feeding area such as a bird table.

Put on plastic gloves. This is necessary because birds can spread disease.

Collect the 'worms' of each of the two types, putting them into separate containers or bags as you do so.

Count how many of each sort of 'worm' is present, then calculate the number of 'worms' that must have been eaten by subtracting the numbers you have counted from the numbers of 'worms' of both varieties that were originally placed in the feeding area.

Each 'generation' of 'worms' contains the same total number (the total population size does not change). However, the ratio of the two types changes in each generation according to how many have been taken by birds, and therefore how many 'worms' of each type are left to 'breed'. Calculate how many new 'worms' now need to be placed on the feeding area as follows:

Total number of 'worms' of both types placed in the last



generation = N
 Number of worms of Type 1 (e.g., brown) collected = a
 Number of worms of Type 2 (e.g., green) collected = b
 Total number of 'worms' collected (of both types) = $a + b = n$

Therefore the number of new 'worms' of each type to be placed is:

New Type 1 (e.g., brown) 'worms' required = $N \times a \div n$
 New Type 2 (e.g., green) 'worms' required = $N \times b \div n$

Place the correct numbers of new 'worms' at random in the feeding area.

Wash your hands.

This collection procedure should be repeated each day or more frequently as required.

Safety guidelines

Birds carry parasites and bacteria such as *Salmonella*. Therefore it is important that students wear disposable plastic gloves when handling food that birds and other animals might have been in contact with and that they wash their hands after doing so.

Lesson 3: Examining and interpreting the results

Once data has been collected for a week or more, students plot the data graphically and interpret their results. The results should be plotted as a graph of the number of each type of 'worm' present vs. time.

Additional investigations

Several variants of the procedure are possible: for example, coloured wool or dyed bread can be used in place of spaghetti baits, or students can act as the 'predators', catching as many 'worms' as possible in a limited time. Allen and his co-workers have suggested various additional ideas in their papers [5, 6], but at the simplest level students may just investigate differential selection of prey on various backgrounds without replacing the baits that have been removed [7].

An important development since the original experiments of this type were conducted is that it is now known that birds' vision is superior to humans' and in particular that many species can see in the UV-A (400–315 nm) range of the spectrum [8]. Students would therefore benefit from trying to appreciate how the world might appear to birds' senses rather than to humans' — although it is difficult to allow for this in the design of the

current investigation.

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Additional resources

From March 2009, the Wellcome Trust's 'Survival Rivals' web site (www.survivalrivals.org) will include additional resources such as an animated game to accompany this protocol.

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