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Day 1: Background and Fieldwork

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ABSTRACT

ADE 2021 description of work to be done on Day 1 of the practical work

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TRADITIONAL AND MODERN APPROACHES TO THE STUDY OF BIODIVERSITY

Background and aims

DNA barcoding is a method that uses the DNA sequence of a chosen locus to identify specimens to species. DNA barcoding can be especially useful when the morphology of an organism is not reliable for determining what species it is - either because no reliable species diagnosis is available, because the specimen comes from a previously undescribed species, or maybe you just are'nt sure what you have. In this project, we will use the DNA barcoding method to provide identification tags for terrestrial arthropods collected from the Blackford Hill area near Kings Buildings.

We will use the mitochondrial cytochrome oxidase 1 (COX1) gene as a barcode marker. Mitochondrial genes are useful for animal DNA barcoding because they are found in all species, are conserved enough to permit isolation, and are also divergent enough to display significant sequence difference between species.

Overview of the exercise

Each student will first collect at least five to ~15 (that's probably a maximum- we don't want to collect more than we can possibly hope to identiy) **different** kinds of arthropod from the woodland or a meadow (we will split you into groups) habitat in the Blackford Hill area (Day 1: Sept 27th). You will identify four of your specimens using DNA barcoding (Day 2: Sept 30); these will be sequenced and analysed via the latest sequencing and bioinformatics technology (in the computer labs, 8 and 11 Nov). Your specimens will also be identified to species as far as is possible using taxonomic keys (Day 3: Oct 4th). Here is the sequence of events:

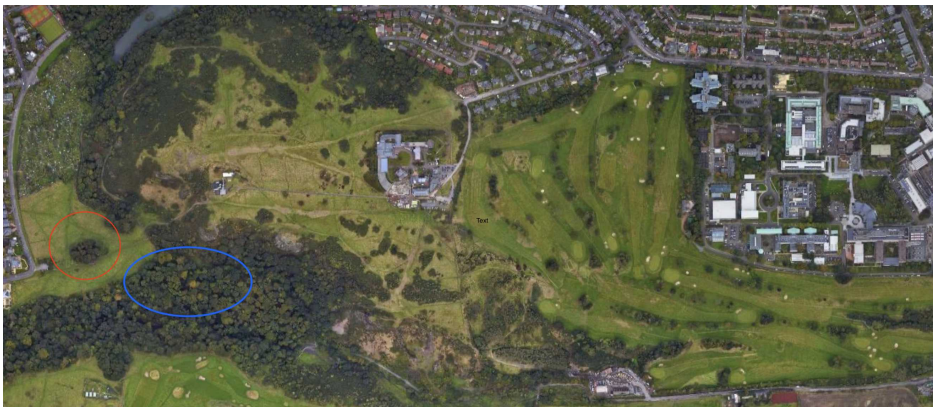
Day 1: Sept 27th. Collect specimens from Blackford hill. Return to the lab organise your specimens for freezing, so that we can later carry our DNA barcoding and morphological taxonomy.

Day 2: Sept 30. Extract DNA from each of your four chosen barcoding specimens and amplify the COX1 gene using PCR

Day 3: Oct 4. Identify your species based on morphological features

(DNA sequence will be acquired from your PCR products using cutting edge Oxford Nanopore sequencing technology. This will be performed by Tom, Andrew, Aine and Danny in between the hands-on labs and the computer practicals on Nov 8 or 11 (depending on which group you are in, again; we will organise groups, splitting up the forest and meadow collection groups). The running of the sequencing device will be live-streamed, and you will be able to view your sequences as the come off the Oxford Nanopore Minion in real time)

Computer practicals: Nov 8, 11. Your sequences will be given to you and you will use online DNA data bases to identify them, compare these to your morphological identifications, and compare diversity in the two habitats.



Proposed sampling sites on Blackford Hill

Day 1: In the field and in Ashworth Lab 1

You will be assigned to either the meadow group or the forest group. Some of the best meadows are circled in red, above. The best forest location is circled in blue.

You will collect specimens of what you think are at least five to 15 different arthropod species. Don't collect more than a few of each putative morphological species, rather aim to collect lots of species. Go for diversity! Sweep your nets, comb through the leaf litter, bang the branches to get the insects to fall off, look under the leaves to find insects hiding, wait by those late flowers to collect a late pollinator, tear apart some rotting wood… Place the specimens in separate jars or containers (or bring them back to the lab in a larger container with some of their environment).

Make careful notes while you are in the Blackford Hill area, and as to where you found the specimens (what kind of habitat, what kind of substrate or plant, etc).

We will only be searching for a short while, so keep busy.

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In the lab

You need to first identify the four specimens you wish to use for DNA barcoding. Sort these four specimens into what you think are different morphological types (“operational taxonomic units”). There are keys available to help with this, but today do not spend too much time trying to get them to species. Taxonomic 'Order' is probably enough. Just make sure you choose **four different** putative species.

You need to choose these four barcoding specimens quickly and freeze them so the tissue will be fresh for DNA barcoding (Day 2). Put each specimen individually into a suitably sized container and label each specimen with your initials and a specimen number (e.g. CW01). Do this with a small piece of paper, using pencil. Also write down where you found it, i.e. what sort of habitat. Put the specimens into a bag, and use permanent marker to label the outside of the bag with your name and "specimens for barcoding". As a back-up, put a strip of paper in each bag labelled with the same info, but written in pencil (pencil will nut run or get rubed off). Get this bag to the tray at the back of the room, where it can be whisked off to the freezer.

Next, do the same with all the other specimens, placing them in another bag with your initials and "Everything else".

Time is limited on this first day, and that is why we are focussing on getting your animals ready for freezing. You will retrieve the four barcoding specimens on day 2 for DNA extraction and PCR. You will retrieve all your specimens (both the barcoding specimens, and everything else) on Day 3 to identify them with dichotomous keys.

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