CamoEvo Lab Practical 1:

Avian Egg Camouflage

Introduction: Camouflage is the primary anti-predator defence for many animals, meaning their survival depends on not being detected or recognised by predators. The eggs of ground-nesting birds are immobile and are often exposed to predators for long periods of time, whilst their parents are absent from the nest. As a result, camouflage is one of the primary driving forces for the colouration and patterns of eggs in different habitats. We also know that birds can choose nest sites that better complement the camouflage of their own eggs.

Example Ground-nesting bird eggs.

In this practical you will be tasked with <u>designing your own experiment</u> to investigate the influence of <u>background features on camouflage</u>. You will do this by first collecting photos from different habitats. Then you will use these photos in a camouflage evolution game which will allow you to simulate the influence of evolution by natural selection on the egg camouflage in different habitats and then measure how their camouflage changed.

The camouflage mechanisms you will measure include colour match, brightness (luminance) match, contrast match, and edge disruption. Edge disruption is a camouflage mechanism found in many species where high-contrast markings interfere with the animal's outline, making it more difficult to detect or identify.

Part 1- Form a Hypothesis

Devising a hypothesis: Before you do anything, you will first need to come up with a hypothesis (or hypotheses) to test. You have the freedom to design you own idea entirely, or you can go with some of the suggestions below. You may also pair up with one other person to share the egg evolution work (though you must do the stats and write-up completely independently). Here are some further suggestions:

- **Keep the background types ("treatments") simple.** It takes approximately 10 generations to produce camouflaged eggs, though this will depend on the backgrounds. So, two backgrounds will probably be the limit per-person, or four if you pair up with one other student. The things that distinguish these backgrounds will be the difference you test.

- **Stick to plausible natural nesting habitats as backgrounds.** The eggs have a limited colour and pattern range to that of ground-nesting birds.

- **24 photos per background.** For each habitat you will need to be able to take 24 photos (so avoid habitats you can't photograph multiple sections of).

- **Manipulation is allowed.** If you want, the difference between habitats can be based on manipulation e.g., placing novel objects (make sure any changes made are reversible, so you don't damage the habitat)

- **Consistent images.** The eggs in the game will spawn in random locations so the appearance of the whole image will be important, so select backgrounds which are consistent in appearance.

- **Stats.** Whatever you come up with, you will need to be able to test your hypotheses using the statistical methods you've learnt during your course or given in the supplementary R code.

- **Observe from Nature.** Try to base your hypothesis and habitats on an observation you've made or read about from nature.

Here are some ideas for hypotheses:

Specialist vs generalist camouflage - ideally requires running three populations: background A (take 12 photos and copy each once to make 24 images), background B (same as A, but with a different background) and background C (12 from A and 12 from B). Effect of anthropogenic "distractors" in the background. e.g., take 24 background photos with and without plastic litter present (or a natural colourful object).

Background pattern size - does the camouflage strategy change with background scale? e.g., take 24 photos of a stony background (such as beach pebbles or gravel) to make background A, then create background B by taking photos of the same substrate from much closer, or use cropping and zooming in on the background A images (making the stones larger relative to the eggs).

Artificial light - take photos of the same patches at night and in the daytime (requires a decent camera, and probably tripod, though good new phones are fine in low light)

Variability in evolutionary trajectories - run the exact same evolutionary algorithm on a single background repeatedly to see how consistent the final strategy is among populations - do they converge or are there different strategies?

Part 2- Habitat Photography Fieldwork

Health & Safety: We will be out on campus for the introduction to the practical and background image photography in all but awful weather conditions, so wear appropriate clothing (warm, waterproof, plus sturdy footwear). Those who can't come to the practical can either use the pre-supplied background images or take their own.

Explore Campus. Have a brief walk around (5-10min) and try to find areas that you think are suitable for comparing / generating your hypothesis. Any background you think will work e.g., fine-grass, mulch, leaf-litter, gravel, but it must be an open, flat area within a 2m radius.

Choose Habitats. Choose habitats or photography methods which relate to your hypothesis.

Take photos. You'll want to take photos from a bird's eye view of the ground, at a consistent height. Make sure the entire photo is in focus, take a few more that 24 for each background to be on the safe side and delete any imperfect images.

Background datasets available. For anyone unable to take their own photos we have a selection of sample backgrounds available to download.

AggregateGrass: Short grass habitat, this forms a continuation of the "Aggregate" series, with backgrounds varying from mostly-aggregate to mostlygrass. The grass is a very uniform colour with stripes.

AggregateLowVeg: This is a gravel/aggregate background with a low level of vegetation cover (part of the aggregate series with the lowest vegetation cover). This is a very complex mix of colours and contrasts between different patches. However, the dominant pattern size from the gravel is consistent.

AggregateMedVeg: This is part of the gravel/aggregate background series with a medium level of vegetation cover. As above, but a higher proportion of vegetation shifts the overall colour and contrast towards the vegetation, with less consistent pattern sizes from the gravel.

LeafLitter: Beech tree leaf-litter. A very high-contrast background where the leaves may be a similar size/shape as the eggs themselves (possibly making them more difficult to find). There are also intense orange colours (which eggs are unlikely to be able to match with their pigment system), but browns they could match well.

Beach Flat: Pebbles/sand with flat ground making it very consistent, taken on a sunny morning. The background pattern size is very consistent.

Beach Furrowed: Pebbles/sand on swanpool with lots of footprints/disruption in the sand creating furrows. It was a sunny morning, so the shaded areas are very blue (lit by the blue sky, creating colour and intensity contrast in the shadows). However there are also consistent pattern sizes from the pebbles.

Beach Seaweed: Pebbles/sand at the high-water mark where there was seaweed. This creates additional colour and luminance contrast, and differences in pattern

Part 3 – Camouflage Evolution Game

Background: Just as the process of evolution by Natural Selection provides a mechanism through which organisms adapt to their environment so too does it provide a mechanism of finding optimal solutions to real world problems. In computer science and research genetic algorithms (GA) can be used optimisation tool inspired by natural selection, where genetic mutation, reproduction and selection are used to create optimal solutions. In the case of this experiment, the problem is 'how eggs should be camouflaged on the habitats you've chosen?' To test this, you will be taking on the role of an ovivorous predator and will attempt to hunt for eggs over multiple generations using a Camouflage Evolution toolbox. With each generation you should be able to see camouflage improve as those with poor camouflage are removed from the population and the survivors produce new individuals through reproduction and mutation.

Copy photos from phone / camera to laptop / PC: Connect your phone / camera to your laptop / PC. Create two folders and copy and paste the photos from each of the backgrounds you chose into their own unique folder. Make sure to label the folders clearly and that you know their location.

Download ImageJ CamoEvo. Download the ImageJ CamoEvo.zip from one of the following links (windows) (mac). Then unzip the file.

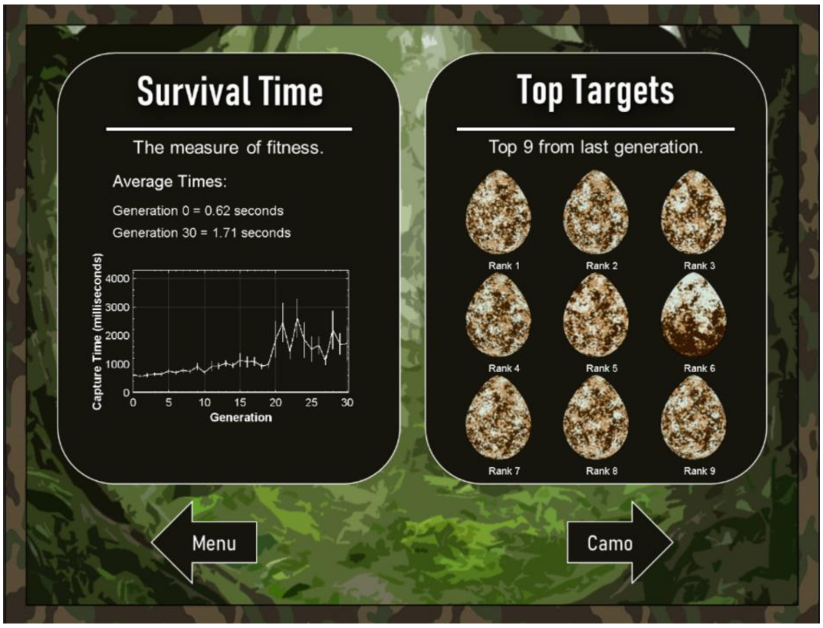
Start ImageJ: Open the ImageJ CamoEvo folder and double click ImageJ.exe

Launch CamoEvo: Click *Plugins -> 1 CamoEvo -> CamoEvo Game.* Click to begin and then skip the tutorial.

Create a New Egg Game: Click New Game and then click get photos. Choose the folder containing one of the sets of photos you took (if you hit cancel, you will need to reopen CamoEvo). You will then be asked to choose what prey you will use. For this practical select Targets and then Egg. You will then be presented with the option to choose advanced settings, select no. Then assign a name to a clear name to your population (no longer than 15 characters).

Playing the Game: Once you have named your population the game will start to generate the first generation of eggs. This may take between 1-4 minutes depending on the speed of your computer. You will then be tasked with finding and capturing all the eggs as quickly as you can by clicking on them. Once all the eggs are found the next generation will develop.

Finishing the Game: Once the game is complete you will be presented with screen asking if you want to view your results or keep going. Click view your results.



Results Preview: You will now be presented with a result preview menu. Feel free to have a look through how capture time, luminance match, colour match, contrast and edge disruption have changed over time. For this practical however you will need to get the results output.

Extracting Results and then sharing them: Following processing of the egg camouflage you can click the button to output the data as a .txt file to a folder of your choice.

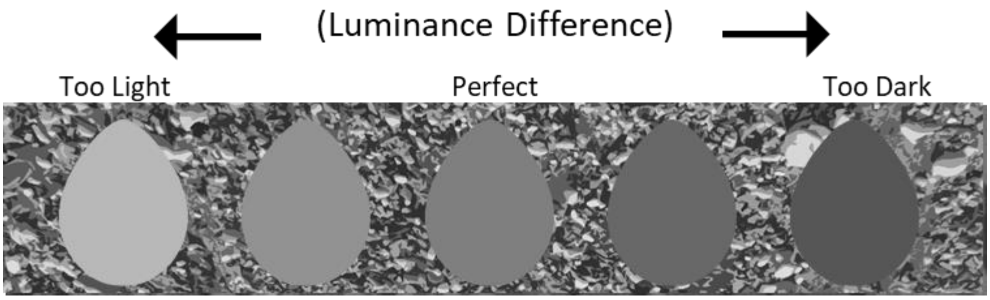
Generation	ID	Capture_Time	Luminance_Difference	Pattern_Difference	Colour_Difference	GabRat_Edge_Disruption
0	Gen0_Mut0_ID22	1033	1.2878	13.0962	11.5133	0.2141
0	Gen0_Mut0_ID16	577	40.4539	8.9096	11.1044	0.1758
0	Gen0_Mut0_ID8	1057	1.1131	6.0624	36.248	0.3357
0	Gen0_Mut0_ID10	1050	5.9535	13.5984	28.6998	0.232

Part 4 – Camouflage Analysis

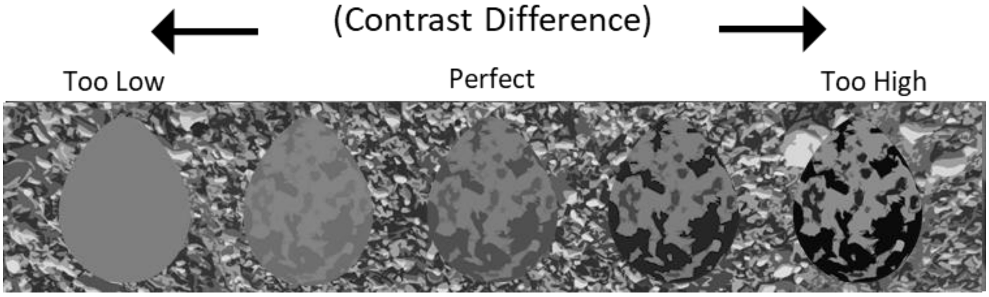
Once you have finished evolving at least 10 generations for all of your habitats you will need to be able to test your hypotheses using statistics.

What do the camouflage metrics actually describe?

- *Luminance Difference* is the difference between the mean luminance (brightness) of the egg compared to its background (here the background is a region within 1 egg-length of the egg itself). This distance is in CIE LAB space (A colour space designed to model human vision). The lower the number the closer the match.

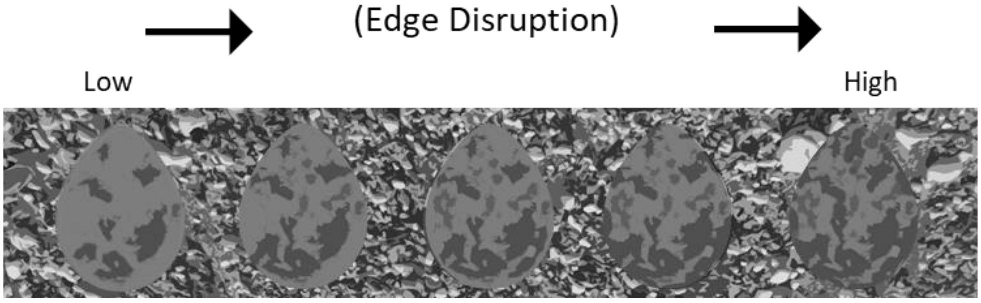


- *Contrast Difference* is the difference between the standard deviation of luminance of the egg compared to its background (e.g. a black & white object will have high contrast, and a grey egg low contrast). The lower the number the closer the match between egg and background contrast.



- *Colour Difference* is the average colour of the egg compared to its background. Specifically, this is the Euclidean distance between “A” (green-red) and “B” (blue-yellow) channels in CIE LAB space. In general numbers below about 2 are impossible for humans to tell apart.

- *Edge Disruption* is the “GabRat” variable described [here](#) (Troscianko J. et al. 2017. Quantifying camouflage: how to predict detectability from appearance. BMC Evolutionary Biology. 17:7). It describes how visible the object’s outline is relative to markings which might disrupt the outline. Higher numbers imply higher levels of disruption (which should make the target more difficult to detect).



Assessment

Your report should take the style of a journal article exploring the link between camouflage evolution and detection, with a focus on the implications for animal behaviour, ecology and evolution. You should read the literature cited here, in the lectures, and elsewhere to determine how our findings fit within the current body of research on this subject. You can focus your report on specific aspects of our findings (e.g. just looking at one or two metrics in greater detail), or explore the broader effects and their relationships.

The write-up’s main text should be a maximum of 1,200 words (excluding title, captions and references). This report makes up 40% of your grade for this module.

Your report should include:

Title: (maximum of 90 characters, including spaces).

Introduction: (don't write an abstract) set the scene for describing this piece of hypothesis-driven research; what hypotheses will you test, how will you test them, and what are your predictions? Your predictions should be justified by coherent logic and/or existing research.

Methods*:* <u>Briefly</u> describe the methods. This can be just two or three sentences saying background photos were taken of habitat X and Y, that you used the CamoEvo tool to generate evolved eggs, and that you then performed these statistics (briefly mentioning the tests for statistical model assumptions). Keep this section short and focussed.

Results*:* include basic descriptive statistics (including sample sizes, number of generations), specific analysis results relevant to your hypotheses (e.g. for each model describe the relationship and give the relevant statistics, such as t, p, z values), and ideally illustrative effect sizes (e.g. "eggs on background A took on average ## times longer to find than those on background B"). Use tables to present numeric results! Tables can make the results more clear, and saves on your word count.

Discussion*:* Do the results fit your predictions, are the relationships and effect sizes biologically meaningful, what are the wider implications for behaviour/ecology/evolution, how could the experiment be improved, what future research might be required? This is your chance to bring everything together and prove you understand your own hypothesis and the implications of your results.

References: List all cited literature in this section (i.e. authors, date, title, journal and DOI or volume & pages). There is no fixed style for this list, but the references must be clear, accurate and consistent. Be cautious of sources which are not pee reviewed. There is no limit on the number of citations as long as they are relevant.
Literature must be cited in the main text using the first author name and date (e.g. "Troscianko *et al.* 2018", *not* numbered citations).

Figures & tables*:* should be included where they are most relevant in the main text. There is no fixed limit to the number of figures, tables, however they *must all* be clear and relevant. Figures can include colour, graphs should plot data so that the distribution of the data is visible (e.g. by plotting all data points in a scatter plot, or by showing error bars/regions/quantiles in other plots). Figure/table captions are limited to 100 words and must not contain information not presented in the main text. This report should be submitted in pdf format via e-Bart & Turn-It-In.

Some things to ponder in the write-up:

- Which variables were the best predictors of reaction/capture time, and how are these likely to affect the behaviour or evolution of ground-nesting birds?
- Do your evolved eggs resemble any real bird eggs?
- Why do you think there is such high variation in the appearance of ground nesting bird eggs?
- How could the experimental design and analysis be improved?
- You could look at the differences between selection pressures (changes over evolutionary time) and also Reaction times separately. These let you ask subtly different hypotheses.

Relevant papers:

- Troscianko, Jolyon, John Skelhorn, and Martin Stevens. 2017. 'Quantifying Camouflage: How to Predict Detectability from Appearance'. *BMC Evolutionary Biology* 17: 7. https://doi.org/10.1186/s12862-016-0854-2.
- Troscianko, Jolyon, Jared Wilson-Aggarwal, Martin Stevens, and Claire N. Spottiswoode. 2016. 'Camouflage Predicts Survival in Ground-Nesting Birds'. *Scientific Reports* 6: 19966. https://doi.org/10.1038/srep19966.
- Stevens, Martin, Jolyon Troscianko, Jared K. Wilson-Aggarwal, and Claire N. Spottiswoode. 2017. 'Improvement of Individual Camouflage through Background Choice in Ground-Nesting Birds'. *Nature Ecology & Evolution* 1 (9): 1325. https://doi.org/10.1038/s41559-017-0256-x.
- Lovell, P George, Graeme D Ruxton, Keri V Langridge, and Karen A Spencer. 2013. 'Egg-Laying Substrate Selection for Optimal Camouflage by Quail'. *Current Biology: CB* 23 (3): 260–64. https://doi.org/10.1016/j.cub.2012.12.031.
- Kilner, R. M. 2006. 'The Evolution of Egg Colour and Patterning in Birds'. *Biological Reviews* 81 (3): 383–406.