Applicant: [redacted]
Organisation: [redacted]
Funding Sought: [redacted]
Funding Awarded: [redacted]

SR18/1042

Evolution and ecological genomics of aposematism in southern two-striped walkingsticks

Aposematism is a defensive strategy in which a warning signal, usually bright distinctive coloration, is coupled with a secondary defence, usually a toxic chemical, that makes the prey unprofitable. Thus, predators that attack the prey experience an unpleasant episode, associate it with the warning signal, and learn to avoid defended prey in the future. The evolution of aposematism has puzzled biologists for over a century. It has evolved repeatedly in many groups of animals, but the initial evolution from well camouflaged, cryptic forms is particularly challenging to explain. New aposematic mutants must face the double disadvantage of being easier to detect and, because of their initial rarity, highly ineffective at educating predators on their unprofitability. Consequently, it is difficult to understand how aposematic individuals can survive and reproduce for long enough for the predators to learn to avoid them. One possible explanation is that the alignment of other factors, such as changes in behaviour or ecological conditions, is necessary for the evolution of aposematism. For example, the relative cost of aposematism can be reduced dramatically if prey evolve behaviours that expose them openly to predators (e.g. sexual displays) or move to a new habitat where camouflage is no longer effective or have different predators.

These hypotheses could ideally be tested by studying species where the evolution of aposematism is ongoing and have both aposematic and cryptic populations. This would allow using genomic data to infer, for example, whether natural selection is acting simultaneously on colour and behaviour in aposematic populations, as well as to asses if that is linked to other traits involved in adaptation to different habitats. Nonetheless, systems of this kind are exceedingly rare. Here, I will use one such species, the southern two-striped walkingsticks (Anisomorpha buprestoides), to study the evolution of aposematism. This species is well-known for its ability to spray a defensive irritating substance. Three main colour forms have been recognised for this species, two of them aposematic (white and orange) and another one cryptic (brown) (fig. 1). The white and orange forms are notably more distinctive, seem to be geographically restricted to sandy environments in central Florida and show little colour variation. However, the brown form has a wider distribution throughout southeastern U.S. (from Louisiana to South Carolina) and encompasses a wide range of coloration ranging from pale yellow to very dark brown (fig. 1). The aposematic forms also show differences in behaviour: they tend to rest on leaves in daylight instead of hiding and bury their eggs instead of dropping them.

Here, I will use genomic data from multiple aposematic and cryptic populations across Florida to evaluate to what extent the differences between them are due to ecological factors and infer the history of the populations, placing emphasis on finding out whether the two aposematic forms have evolved independently. Subsequently, I will identify genomic regions under selection will test if selective genomic changes are associated with changes in colour, behaviour, or habitat.

PRIMARY APPLICANT DETAILS

Title	[redacted]
Name	[redacted]
Surname	[redacted]
Organisation	[redacted]
Website (Work)	[redacted]
Tel (Work)	[redacted]
Email (Work)	[redacted]
Address	[redacted]

CONTACT DETAILS

Role	**Referee 2**
Title	[redacted]
Name	[redacted]
Surname	[redacted]
Email (Work)	[redacted]

CONTACT DETAILS

Role	**Referee 1**
Name	[redacted]
Surname	[redacted]
Email (Work)	[redacted]

Section 1 - Eligibility

Please answer the questions below to determine if you are eligible for this scheme

Does the project form part of a degree/thesis/fellowship?

No

Is this project a component of a larger already funded study?
(We accept there may be some additional funding from other streams, however the BES contribution should constitute for the majority of the project funding)

No

Please note we do not accept resubmissions of the same project. Applications will be rejected without being sent out for review if they are re-submissions of a proposal rejected in a previous round, or if they represent only a minor revision of such a proposal (for example, with a modified experimental design). As a guide, in order to be significantly different, at least 80% of objectives & activities should be different to the original proposal.

Using the above statement, is this project a resubmission?

No

Section 2 - Contact Details

PRIMARY APPLICANT DETAILS

Title	[redacted]
Name	[redacted]
Surname	[redacted]
Organisation	[redacted]
Website (Work)	[redacted]
Tel (Work)	[redacted]
Email (Work)	[redacted]
Address	[redacted]

CONTACT DETAILS

Role	Referee 2
Title	[redacted]
Name	[redacted]
Surname	[redacted]
Email (Work)	[redacted]

CONTACT DETAILS

Role	Referee 1
Name	[redacted]
Surname	[redacted]
Email (Work)	[redacted]

GMS ORGANISATION

Type	Organisation
Name	[redacted]
Phone (Work)	[redacted]
Email (Work)	[redacted]
Website (Work)	[redacted]
Address	[redacted]

If you do not have a current organisation (i.e. you are an independent researcher/retired), please provide your preferred contact address above and select the checkbox to the right. Unchecked

BES Membership Number

[redacted]

Section 3 - CV

Education History

Please note if you do not know the exact day, select the 1st day of the month e.g. March 2005: 01/03/2005

Start Date	End Date	Qualification	Organisation	Additional Info
[redacted]	[redacted]	M.Sc. in Biology	[redacted]	*No Response*
[redacted]	[redacted]	PhD in Genetics	[redacted]	PhD thesis: [redacted]
No Response	*No Response*	*No Response*	*No Response*	*No Response*

Employment History

Please note if you do not know the exact day, select the 1st day of the month e.g. March 2005: 01/03/2005

Start Date	End Date	Position	Organisation	Additional Info
[redacted]	[redacted]	Predoctoral Research Fellow [redacted]	[redacted]	*No Response*

[redacted]	[redacted]	Predoctoral [redacted]	[redacted]	*No Response*
[redacted]	[redacted]	Research Staff Training [redacted]	[redacted]	*No Response*
[redacted]	[redacted]	Postdoctoral Research Associate	University of [redacted]	*No Response*
[redacted]	[redacted]	[redacted] Zoology Fellow	University of [redacted]	*No Response*
[redacted]	[redacted]	[redacted] Early Career Fellow	University of [redacted]	*No Response*

Research Grants/Fellowships Awarded

Date	Details	Amount Awarded (£)	Additional Info
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Conference Participation
Please list a maximum of 3 most recent and relevant conferences

Conference Name	Details
51 Population Genetics Group Meeting, Bristol (UK), 6-5 January 2018	[redacted]
BES Macro 2017, London (UK), 5-7 July 2017	[redacted]
XXV International Congress of Entomology, Orlando (USA), 25-30 September 2016	[redacted]

Other Awards/Achievements/Skills

2016	[redacted]
2018	[redacted]
2018	[redacted]
2018	[redacted]
2016	[redacted]
2014	[redacted]
2013	[redacted]
2009	[redacted]

Summary of Publication Record

[redacted]

Professional Membership

European Society for Evolutionary Biology (ESEB), Society for the Study of Evolution (SSE), Systematics Association, British Ecological Society (BES), The Genetics Society, Spanish Society of Evolutionary Biology (SESBE), Society of Spanish Researchers in the United Kingdom (SRUK), Catalan Biology Society (SCB).

Additional Information

Supervision:
[redacted]

[redacted]

[redacted]

[redacted]

Services to the scientific community:
I have acted as reviewer for several journals in the fields of genomics and evolutionary biology ([redacted]

Section 4 - Project Details and Keywords

NB: The Total Project Cost and Amount Requested will be added automatically from the figures you provide on the budget page.
Once you have completed your budget, you will be required to come back into this page, ensure the figures are correct, and save the page.

Summary Project Details

Total project cost: £	**Amount requested from BES: £**
4,991.00	4,991.00

Please read our Bulletin article for guidelines on writing an effective lay summary.

Project title:

Evolution and ecological genomics of aposematism in southern two-striped walkingsticks

Project lay summary:

Aposematism is a defensive strategy in which a warning signal, usually bright distinctive coloration, is coupled with a secondary defence, usually a toxic chemical, that makes the prey unprofitable. Thus, predators that attack the prey experience an unpleasant episode, associate it with the warning signal, and learn to avoid defended prey in the future. The evolution of aposematism has puzzled biologists for over a century. It has evolved repeatedly in many groups of animals, but the initial evolution from well camouflaged, cryptic forms is particularly challenging to explain. New aposematic mutants must face the double disadvantage of being easier to detect and, because of their initial rarity, highly ineffective at educating predators on their unprofitability. Consequently, it is difficult to understand how aposematic individuals can survive and reproduce for long enough for the predators to learn to avoid them. One possible explanation is that the alignment of other factors, such as changes in behaviour or ecological conditions, is necessary for the evolution of aposematism. For example, the relative cost of aposematism can be reduced dramatically if prey evolve behaviours that expose them openly to predators (e.g. sexual displays) or move to a new habitat where camouflage is no longer effective or have different predators.

These hypotheses could ideally be tested by studying species where the evolution of aposematism is ongoing and have both aposematic and cryptic populations. This would allow using genomic data to infer, for example, whether natural selection is acting simultaneously on colour and behaviour in aposematic populations, as well as to asses if that is linked to other traits involved in adaptation to different habitats. Nonetheless, systems of this kind are exceedingly rare. Here, I will use one such species, the southern two-striped walkingsticks (Anisomorpha buprestoides), to study the evolution of aposematism. This species is well-known for its ability to spray a defensive irritating substance. Three main colour forms have been recognised for this species, two of them aposematic (white and orange) and another one cryptic (brown) (fig. 1). The white and orange forms are notably more distinctive, seem to be geographically restricted to sandy environments in central Florida and show little colour variation. However, the brown form has a wider distribution throughout southeastern U.S. (from Louisiana to South Carolina) and encompasses a wide range of coloration ranging from pale yellow to very dark brown (fig. 1). The aposematic forms also show differences in behaviour: they tend to rest on leaves in daylight instead of hiding and bury their eggs instead of dropping them.

Here, I will use genomic data from multiple aposematic and cryptic populations across Florida to evaluate to what extent the differences between them are due to ecological factors and infer the history of the populations, placing emphasis on finding out whether the two aposematic forms have evolved independently. Subsequently, I will identify genomic regions under selection will test if selective genomic changes are associated with changes in colour, behaviour, or habitat.

Project start date: 14/11/2018

Project end date: 14/02/2020

Project country:

United Kingdom

We have chosen a selection of key words which cover the breadth of the ecological research we fund to link the ecological content of an application to the most appropriate member of the BES Review College.

Your selections will determine which reviewers are asked to review your application. Please select carefully.

Please choose three from the following:

- [x] Biodiversity
- [x] Ecological Genetics
- [x] Evolutionary ecology

Section 5 - Grant Specific Questions

Please provide a project description

This should include:

a) background and rationale
b) the question or hypothesis to be tested
c) an outline of the methods to be use
d) expected outputs
e) expected timescales

Please capitalise all headings

Background and rationale:
The origin and evolution of aposematism has puzzled biologists for a long time. It is a recurrent defensive strategy that has evolved repeatedly in many groups of animals and yet the initial evolution from cryptic forms is particularly challenging to explain. It is difficult to understand how the few initial aposematic mutants can survive and reproduce for long enough for the predators to learn to avoid them. One possible explanation is the alignment of other factors, such as changes in

behaviour or ecological conditions, is necessary for the evolution of aposematism. For example, the relative cost of aposematism can be reduced dramatically if prey evolve behaviours that expose them to predators or colonize new habitats where camouflage is no longer effective.

Species where both cryptic and aposematic forms are found are ideal to advance in the understanding of the evolution of aposematism, but are exceedingly rare. Here, I will study the evolution of aposematism in one of such species, the southern two-striped walkingsticks (Anisomorpha buprestoides). This species can spray a defensive irritating secretion and three main colour forms have been recognised: white, orange and brown (fig. 1). The white and orange forms are aposematic and may have evolved in parallel. They are geographically restricted to sandy habitats in central Florida and show little colour variation, bury their eggs and rest on plants in daylight. However, the brown form has a wider distribution from Louisiana to South Carolina (U.S.), encompasses a wide range of colour variation (fig. 1), drop their eggs and hide during the day.

Here, I will follow a population genomics approach to dissect the evolution of aposematism in A. buprestoides. I will first use genome-wide data from multiple populations to study the structure and history of the populations, placing emphasis on finding out whether the structure is driven by particular ecological factors and whether the aposematic forms have evolved independently. Subsequently, I will identify genomic regions under divergent selection between cryptic and aposematic populations and test to what extent genetic change is associated with simultaneous changes in colour, behaviour, or habitat.

Questions:
1) Is the population structure shaped by ecological factors? Did white and orange aposematic forms evolve independently?
2) Are genomic regions under selection associated with differences in colour, behaviour, or habitat?

Methods:
I will sample ten different populations across Florida: eight of them from within 100km around the areas where the two aposematic morphs are found (Archbold Biological Station and Ocala National Forest), and two from further North and South extremes. I will register information on the habitat and behaviour during the sampling and will take standardised photos to measure colour. Already available samples of the closely related species A. ferruginea and A. paromalus will be used as outgroups for genetic analyses. DNA extractions will be carried out using spin-column kits. Genotype-by-sequencing (GBS) libraries and sequencing will result in ~50,000 loci. After quality control and bioinformatics processing, I will perform population structure, phylogeographic, and demographic modelling analyses to infer the history of the populations. Secondly, I will use genome-wide scans to identify loci under selection and/or associated with colour, behaviour, or habitat.

Outputs:
Publication in a peer-reviewed journal, such as Current Biology or Molecular Ecology. It will also provide crucial information for further studies on other ecological and genomic aspects of the evolution of aposematism in this system.

Timescale:
November 2018: field sampling.
December 2018: DNA extractions.
January-February 2019: GBS library preparation and sequencing.
Mar 2019: quality control, reference sequence assembly, alignment.
April-June 2019: population structure analyses, phylogeography and demographic modelling.
July-August 2019: genome-wide scans.
September-November 2019: write manuscript draft.

What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?

Risk: Insect collection in subtropical conditions (high temperature, solar radiation)
Management: Wear appropriate clothes (e.g. sun hat), drink water, use sunscreen.

Risk: Studied species can spray defensive secretion
Management: Handle with care, use goggles and gloves whenever possible.

Risk: Preservation of samples in ethanol
Management: Use gloves and fume hood whenever possible.

Risk: Lab work (DNA extractions)
Management: The Molecular Ecology Lab at the Department of Animal and Plant Sciences, University of Sheffield, will provide the necessary support, training and induction and has well-established health and safety protocols and risk assessments in place.

Please identify any ethical considerations that may arise from the project.

There are no ethical considerations associated to the project.

Please provide details of agreed collaborations and project partners that will facilitate the proposed project:

I will apply for a stay at the [redacted] which will provide accommodation for large part of the sampling and is equipped with a entomological material for collecting samples and a multi-purpose lab with equipment for for taking standardised photos and preparing samples. Upon contact with the Facilities Coordinator, I have been informed that applications are usually processed in less than a week.

Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:

[redacted] has a strong expertise in genomics of local adaptation, speciation and evolution of colour with renowned researchers such as [redacted]. I will undertake the hands-on laboratory work at our Molecular Ecology Lab facility. The Facility provides all the equipment that we require plus basic consumables (including plasticware, disposables such as gloves, agarose, buffers, primers, PCR and DNA extraction reagents and pipettes), as well as basic technical support to cover equipment maintenance and replacement, consumables ordering, induction and basic training in the use of the laboratory. The Facility will support all the sample handling and preparation, including DNA extraction and QC. However, the Facility does not provide some specialist items such as DNA extractions kits, for which I request a separate specific budget. DNA samples will then be shipped to the Genomic Sequencing and Analysis Facility (GSAF) [redacted] where GBS library preparation and sequencing will be performed. Subsequent computational analyses will be carried out using the Iceberg and ShARC clusters of [redacted], which are free to use for all researchers.

Please provide details of necessary permits/licences obtained, if applicable:

Non-commercial insect collection in United States is permitted in public land outside protected areas, such as National Forests.

Please indicate how you will ensure the reach and impact of the project for both academic and non-academic audiences:

This project will result in a high profile publication in a scientific journal (target journals: Current Biology, Molecular Ecology). I will endeavour to present the work to the academic audience via poster or oral presentation at the Annual Meeting of the British Ecological Society and/or of the meeting of the Ecological Genetics Special Interest Group. I will engage in local outreach events, specifically targeting a presentation/activity to be part of the Department of Animal and Plant Sciences programme at the [redacted] Festival of Science and Engineering and/or the British Science Week.

How do you intend to make your research data publically available?

Results will be published under Open Access licences. Sequence data will be deposited in the NCBI Sequence Read Archive (SRA). All other data will be deposited in ORDA, the University of Sheffield Research Data Catalogue and Repository. Code used for analyses will be deposited on a bitbucket repository.

Please provide details of any published papers you are involved in relevant to this project:

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Have you previously applied for a grant from the BES?

No

If you would like to upload a document in support of your application please attach using the control below. Please make sure you reference these in your text.

NB: A maximum of 1 file can be uploaded. Please only upload files essential to the application. Files with additional text to supplement word limits will not be considered and may make your application ineligible.

[redacted]
07/09/2018
21:24:56
pdf 906.47 KB

Section 6 - Budget

Please note all budgets must be provided in Great British Pounds (GBP)

Equipment/Consumables

Item	Quantity	Description	Total Cost
QIAGEN DNEasy Blood & Tissue kit (50 samples)	3	Tissue DNA extraction kit	£500.00
Genotype by Sequencing (GBS)	104	GBS library preparation and sequencing at the Genomic Sequencing and Analysis Facility (GSAF) of the University of Texas, Austin: HiSeq2500, PE 125bp reads, ~50,000 loci at 5-10x.	£3,000.00
No Response	*No Response*	*No Response*	*No Response*
		Overall Equipment Cost:	**£** 3,500.00

Personal Travel/Accommodation/Subsistence

Description	Total Cost
No Response	*No Response*
No Response	*No Response*
No Response	*No Response*
Overall Personal Travel Cost:	**£** 0.00

Field Travel/Accommodation/Subsistence

Description	Total Cost
Travel [redacted] (flight + trains)	£500.00
Car rental - 10 days	£250.00
Accommodation in Florida - 10 days	£500.00
Overall Field Travel Cost:	**£** 1,250.00

Employment
(note only casual, short term assistance will be considered)

Position	Description of role	Rate & Duration of employment	Total Cost
No Response	*No Response*	*No Response*	*No Response*
No Response	*No Response*	*No Response*	*No Response*
No Response	*No Response*	*No Response*	*No Response*
		Overall Employment Costs:	**£** 0.00

Other
(note requests to cover conference attendance or publication costs will not be considered)

Item	Description	Total Cost
Postage	Shipping of samples and DNA to GSAF for library preparation and sequencing	£100.00
Molecular Ecology Lab use	2 day-access to the Molecular Ecology Lab facility at the Department of Animal and Plant Sciences of the University [redacted] for DNA extractions	£141.00
No Response	*No Response*	*No Response*
	Overall Other Costs:	**£** 241.00

Total project cost:
£
4,991.00

Amount Requested from BES
£4,991.00