	Experiment title:	Experiment number:
	Using structural sorting to isolate the genetic instructions responsible for structural colour in Heliconius Butterflies	LS2720
Beamline: ID02	**Date of experiment**: from: 28th September 2017 to: 2nd October 2017	**Date of report**: 26th February 2020
Shifts: 9	**Local contact(s)**: Dr Thomas Zinn	*Received at ESRF:*

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Report:

The aim of this continuation proposal was to examine the differences in the structural colour elements of hybrid (crosses) Heliconius butterflies, and link this to the genetics that controls these structures. We wanted to use ultra-small angle x-ray scattering (USAXS) to map regions of the Heliconius wing hybrids and then quantitatively compare the different scattering patterns and hence the nanostructures and their hierarchical arrangement, which are ultimately responsible for the structural colour and appearance of the wing. This we hoped would allow us to pinpoint the genetic code responsible for controlling the structural colour, this is currently unknown.

We received great technical support and assistance from our beam scientist Dr Zinn. The machine ID02 works fantastically well and the continuous data reduction script allowed us to assess the quality of the data we were collecting as we went along. An example dataset is shown in figure 1, along with the particular structural features.

Bright, highly reflective iridescent colours can be seen across nature and are produced by the scattering of light from nanostructures. Heliconius butterflies have been widely studied for their diversity and mimicry of wing colour patterns. Despite iridescence evolving multiple times in this genus, little is known about the genetic basis of the colour and the development of the structures which produce it. Heliconius erato can be found across Central and South America, but only races found in western Ecuador and Colombia have developed blue iridescent colour. Here, we use crosses between iridescent and non-iridescent races of H. erato to study phenotypic variation in the resulting F2 generation. Using measurements of blue colour from photo-graphs, we find that iridescent structural colour is a quantitative trait controlled by multiple genes, with strong

evidence for loci on the Z sex chromosome. Iridescence is not linked to the Mendelian colour pattern locus that also segregates in these crosses (controlled by the gene cortex). Small- angle X-ray scattering data show that spacing between longitudinal ridges on the scales, which affects the intensity of the blue reflectance, also varies quantitatively in F crosses.

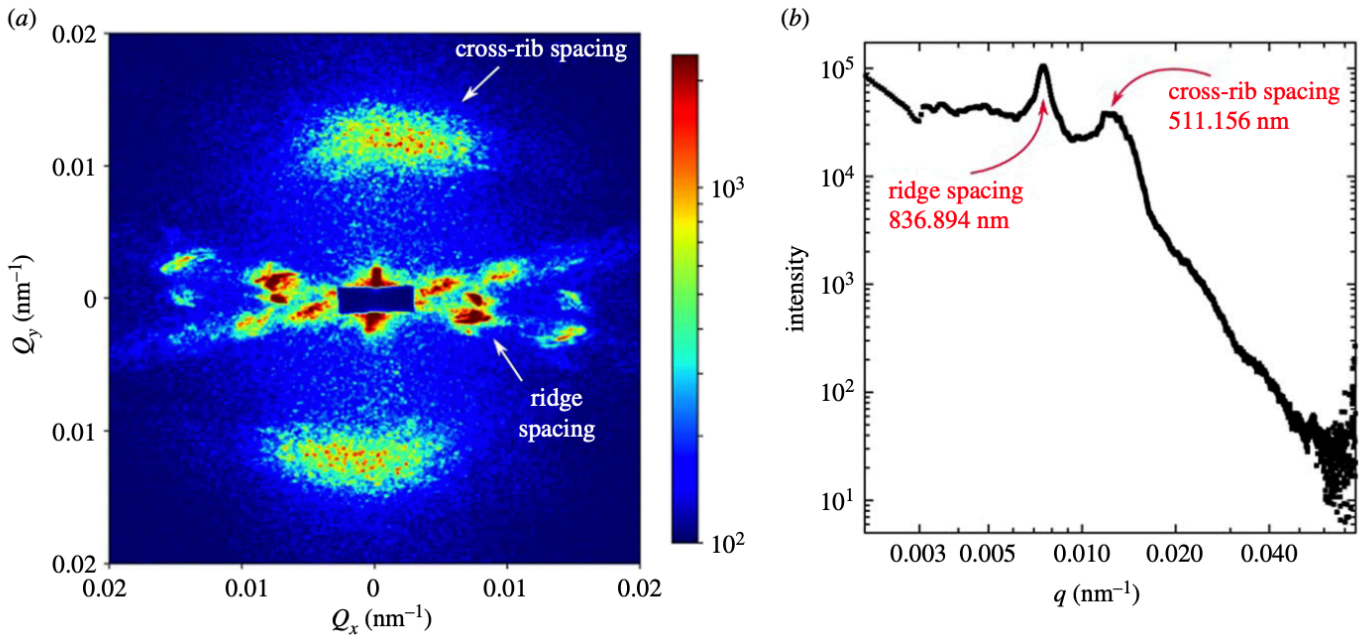

Figure 1. Representative SAXS patterns for a single frame of a male H. erato cyrbia parent. (a) The 2D pattern reveals approximately perpendicular scattering intensity from scale features. From their orientation, length scales of the scattered intensity and previous interpretations, we infer that they correspond to the spacing between ridges and cross-ribs. (b) Full azimuthal integration of the scattered intensity as a function of the magnitude of the momentum transfer vector q. The peaks corresponding to ridge and cross-rib spacing are indicated together with the length scales in real space.

Conclusions

Crosses are ideal for investigating the genetic basis of colour and pattern as traits will segregate in following generations. Crossing iridescent and non-iridescent H. erato has allowed us to quantify variation in the colour and determine that it is sex-linked and controlled by multiple loci in the genome and not just a single gene.

This work was published in reference 1 and will also form part of further planned papers and the thesis of a Sheffield student Juan Enciso-Romero.

1. M. N. Brien et al., Phenotypic variation in Heliconius erato crosses shows that iridescent structural colour is sex-linked and controlled by multiple genes. Interface Focus. 9, 20180047–12 (2019).