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Museum epigenomics as a Toolbox in Evolutionary Research

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Summary

Epigenomics is among the fastest-growing and most dynamic fields in the life sciences. It enhances our understanding of the molecular mechanisms underpinning taxonomically and evolutionarily relevant phenotypic variation by exploring how epigenetic signatures, such as DNA methylation, may act as important drivers of phenotypic diversity. Interestingly, these modifications can be environmentally triggered and may mediate changes in gene expression levels within an individual, thereby allowing a single genotype to show remarkable phenotypic variation depending on its surrounding environment (phenotypic plasticity). These epigenetic processes may at least partly mirror the sometimes exceptional, and taxonomically confusing, intraspecific morphological variation observed in nature and museum collections. Despite the growing awareness of the unprecedented opportunities natural history collections have to offer and the increasing rate at which they are being mobilized for scientific purposes, to date natural history collections remain largely ignored for epigenomic research. While the Royal Belgian Institute of Naturals Sciences (RBINS) has a longstanding track record of conducting evolutionary research on both museum collections and contemporary samples, in-house epigenomic expertise to explore these resources is currently lacking at the RBINS. As an overarching goal of this project we intend to bring new technological and analytical epigenomic expertise into the RBINS in order to strengthen and stimulate collection-based evolutionary research.

Notwithstanding a variety of epigenetic markers have been described and studied, cytosine methylation seems the most promising candidate for epigenomics studies of museum collections as this sort of epigenetic modification is expected to remain stable over extensive time periods. We here applied whole genome and reduced representation bisulphite sequencing to detect such DNA methylation patterns. During the bisulfite reaction of DNA, only unmethylated cytosines are converted to uracils, while methylated cytosines remain unaffected by the bisulphite treatment.

We explored methylation patterns in museum samples stored at suboptimal conditions (i.e. sampled more than three decades ago and stored on ethanol at room temperature) and compared them with methylation profiles obtained from samples preserved under optimal conditions (stored at -80°C, flash-frozen using liquid nitrogen upon sampling). Frequency plots of the percentage of methylated bases in ethanol preserved samples were comparable to those preserved in optimal conditions and similar trends were found for methylation estimates per base pair. To explore to what extent inferior storage conditions could lead to an up- or downwards bias in methylation calling, we extracted the maximum and minimum methylation rates per site from the methylation database of optimal preserved samples. These upper and lower limits were set as the expected methylation range per site. Overall, only 2.89% and 3.04% of all sites were highlighted as respectively hypermethylated (above the range) and hypomethylated (below the range) in ethanol preserved samples, while 94.1% of the sites showed methylation values well within the expected range. Moreover, these hyper- and hypomethylated sites were randomly distributed across sites and individuals. In conclusion, we found no evidence that less optimal preserved museum samples showed clear signatures of hypo- or hypermethylation as methylation rates of these samples seemed to remain comparable to those

preserved under the highest preservation standards. Hence, it seems that methylation patterns remain unaffected by inferior preservation conditions and these type of collections therefore remain a valuable resource in future museum epigenomic studies.

We here applied this newly gained expertise to investigate the role of DNA methylation in the adaptive evolution of *Calosoma* beetles and to evaluate the relative contributions of genetic determination and phenotypic plasticity to morphological adaptations in *Tectarius* snails.

RBINS has a longstanding track record of conducting evolutionary research on adaptive radiations with a specific focus on those occurring at the Galápagos. A most intriguing radiation is those of the *Calosoma* beetles, where representatives of this genus radiated repeatedly into a highland and lowland ecotype along an altitudinal gradient replicated on all major islands. At volcano tops, these species show a strong reduction in size of traits related to dispersal (e.g. wing size reduction). Here we investigate the role of methylation in adaptive evolution by exploring whether previously identified genomic regions under genetic selection are enriched for differentially methylated regions. Unfortunately, no methylation signature could be detected in any of the samples. This may indicate that either the genome of beetles is not methylated at all and therefore does not play a crucial role in differential wing development or that key genes are not methylated in adults (this study), but rather in other developmental (i.e. larval) stages. Investigation of another epigenomic technology aimed at characterizing open chromatin structures showed however considerable promise.

In another case study, we investigated the shell polymorphism of the planktonic developing periwinkle *Tectarius striatus* in the Azores and Cape Verde. Here two shell morphotypes of *Tectarius striatus* co-occur at a microgeographical scale, but display a non-random distribution across the landscape. The 'nodulose' morphotype has a highly sculptured and lighter shell and predominates at wave-sheltered areas. In contrast, the 'smooth' morphotype is most commonly observed at wave-exposed areas. These habitat-related shell morphologies resemble different functional strategies. In this study we explored both genomic and epigenomic variation to identify the underlying molecular drivers of the observed shell variation. While genomic analyses revealed a clear island-based population structure, no morphotype-specific genomic differences were detected. In contrast, epigenomic variation could discriminate between the two shell morphotypes, with several sites showing significant differential methylation. Together, these findings suggest that shell morphology is an epigenetically determined plastic trait.

In conclusion,

- Epigenomic wet-lab protocols and bioinformatic pipelines were successfully established for the analysis of museum specimens and can now be readily disseminated to researchers at RBINS and other institutions.

- Specimens preserved under suboptimal conditions can nonetheless yield high-quality epigenomic data, confirming their value as a resource for museum-based epigenomic research. These results justify and encourage further investment in the exploration of a wider diversity of museum collection types.

- While the *Calosoma* genome appears to lack DNA methylation, the investigation of open chromatin structures represents a promising alternative avenue for epigenomic analysis.

- Adaptive shell morphology in snails showed no detectable genetic basis, but instead appears to be primarily driven by epigenetic mechanisms.