A novel understanding of ASIP in *Marmota caligata*.

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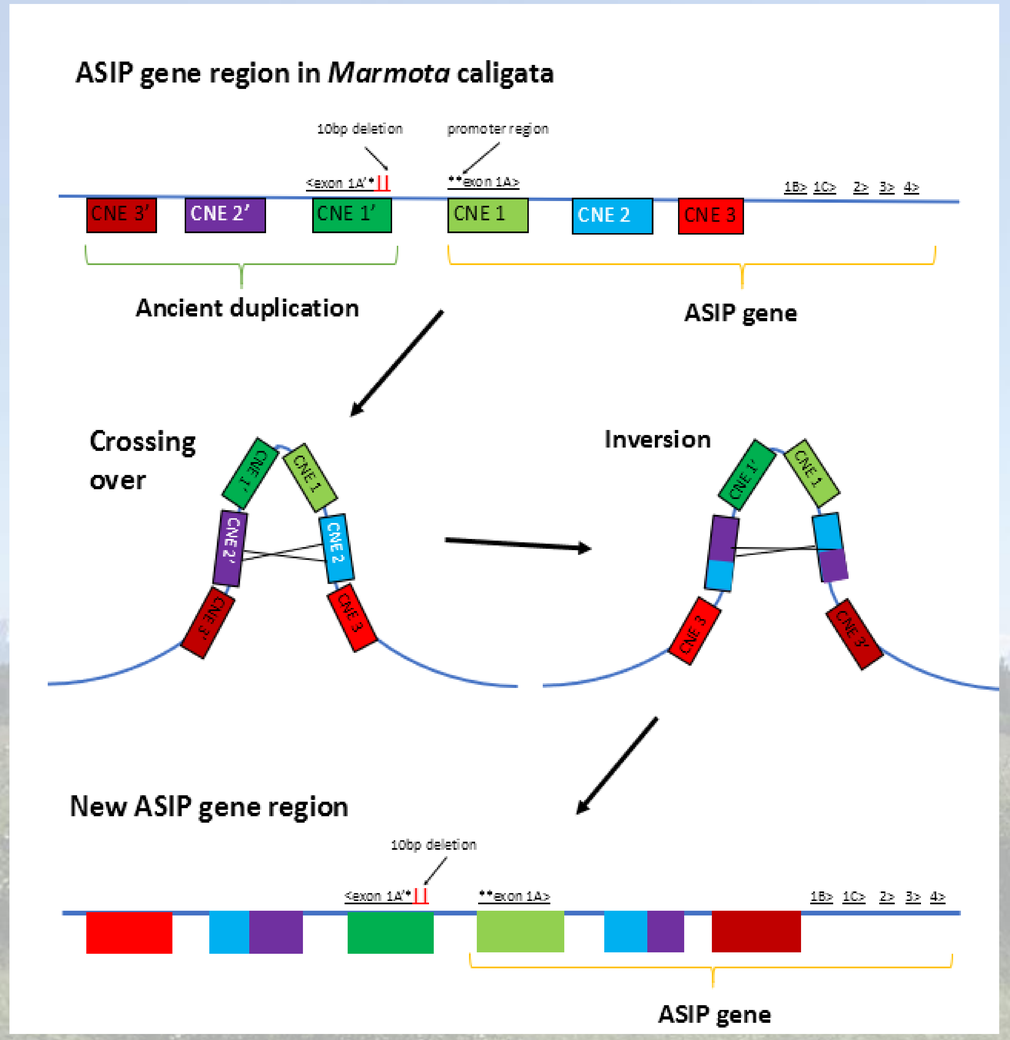
Introduction

The agouti signaling protein (ASIP) gene is questionably arranged and suggests that conserved noncoding elements (CNEs) have mediated at least one inversion. This study aims to determine the correct sequence of ASIP in the hoary marmot so that we can evaluate the functional consequences of the putative mutations. This protein has several pleiotropic effects, but is best known for interacting with Melanocortin-1 receptors (MC1R) to signal a secondary messenger system. This cascade initiates the production of pheomelanin, which is attributed to light fur color[4]. The current ASIP gene region assembly for *Marmota caligata* has several assembly gaps and is questionably arranged due to inversions of the conserved noncoding elements (CNEs), which contain the promotor region and exon 1A. This study investigates the ASIP gene assembly gaps, as well as maps the CNEs contrary to how the assembly portrays.

Methods

I designed primers using Geneious to target the assembly gaps in the ASIP gene among 48 *Marmota caligata* individuals. Amplicons were sequenced using a combination of Sanger and minION technology. I analyzed, assembled, and interpreted the sequences in Geneious to determine how ASIP is truly assembled, as well as fill in the assembly gaps.



Conclusions

The newly assembled sequences established in this study suggest that the current assembly is incorrect. Furthermore, these data suggest a high likelihood an inversion occurring among the duplicate CNEs found in this species. Because the inverted CNEs are similar to the CNEs responsible for proper expression of ASIP, the region containing all the CNEs could create a hairpin structure and result in unequal crossing over.

Next steps

1. I will perform the same study with the hoary marmot's sister species, the critically endangered Vancouver Island marmot, which may be melanistic because this region was re-inverted but carried a new mutation back to the promoter region of ASIP
2. I will also study the pleiotropic effects of a conspicuously non-functioning ASIP in *Marmota vancouverensis*. Physiological differences found in a mammal species with little to no expression of ASIP could be used to model ASIP deficiencies in humans.

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