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This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement
This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (https://scholarworks.iu.edu/dspace/handle/2022/7911).

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Candidate genes and rapidly evolving migratory behavior in the genus *Junco*

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INTRODUCTION

- Despite extensive experimental evidence indicating a strong genetic basis underlying variation in the occurrence, timing, and distance of avian migration[1], very little is known about the specific genes that regulate migratory behavior.
- Tandem repeat (TR) variation in functional regions of the genome can code for phenotypic variation and facilitate evolution [2].
- Variation in TR length of *ADCYAP1* gene (neuropeptide that affects melatonin secretion) has been associated with variation in migration distance in black caps (*Sylvia atricapilla*) [3].
- Another candidate gene, *CLOCK* (a transcription factor involved in circadian rhythm regulation) is not associated with black cap migration distance, but shows latitudinal clines in other songbirds [4].
- To test the generality of these findings, we investigated whether TR polymorphisms in *ADCYAP1* and *CLOCK* help to explain the highly variable migratory behavior of the North American sparrow genus *Junco* (Table1) that includes latitudinal, altitudinal, and non-migratory subspecies and populations.

QUESTIONS

- Do frequencies of *ADCYAP1* or *CLOCK* TR polymorphisms co-vary with population migratory phenotype?
- Do *ADCYAP1* or *CLOCK* TR polymorphisms correlate with individual migratory restlessness?

EXPERIMENTAL DESIGN

- DNA collected from 8 populations with variable migratory behavior (Table1).
- Birds from two recently diverged populations were raised in a common garden and tested for migratory restlessness.
- Amplicon length measured with ABI 3730 and Peak Scanner in mulitplex PCR.

FIGURE 1: Sampled *Junco* populations and their migratory disposition

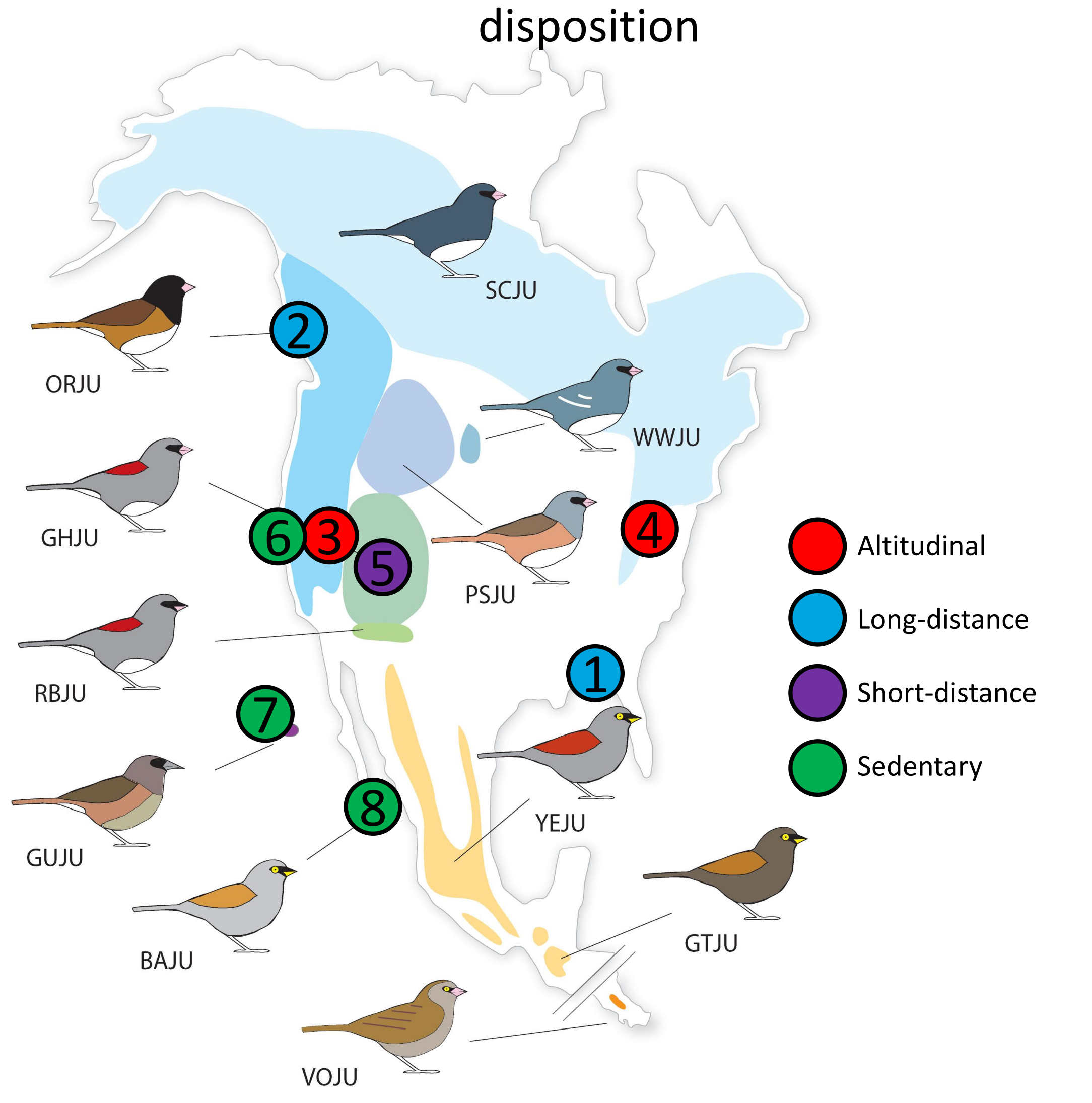


TABLE 1: Sampled populations

# on map	SPECIES	SUBSPECIES	# individuals sampled	BREEDING GROUNDS	WINTERING GROUNDS	MIGRATORY PHENOTYPE
1	J. hyemalis	hyemalis	18	Northern U.S./Canada	Mississippi	long-distance latitudinal
2	J. hyemalis	oreganus	15	British Columbia, Canada	?	long-distance latitudinal
3	J. hyemalis	thurberi	32	California, mountains	California, San Diego county	Altitudinal, facultative
4	J. hyemalis	carolinensis	23	Virginia, mountains	Virginia, valleys	altitudinal, facultative
5	J.hyemalis	caniceps	28	Utah, mountains	Utah, lower elevations and latitudes	Short range, altitudinal
6	J. hyemalis	thurberi	34	California, San Diego, UCSD campus	California, San Diego, UCSD campus	sedentary
7	J. insularis	insularis	18	Guadalupe Island	Guadalupe Island	sedentary
8	J. phaenotus	bairdi	24	Baja California, mountains	Baja California, mountains	sedentary

FIGURE 2: Frequency distribution of alleles in populations

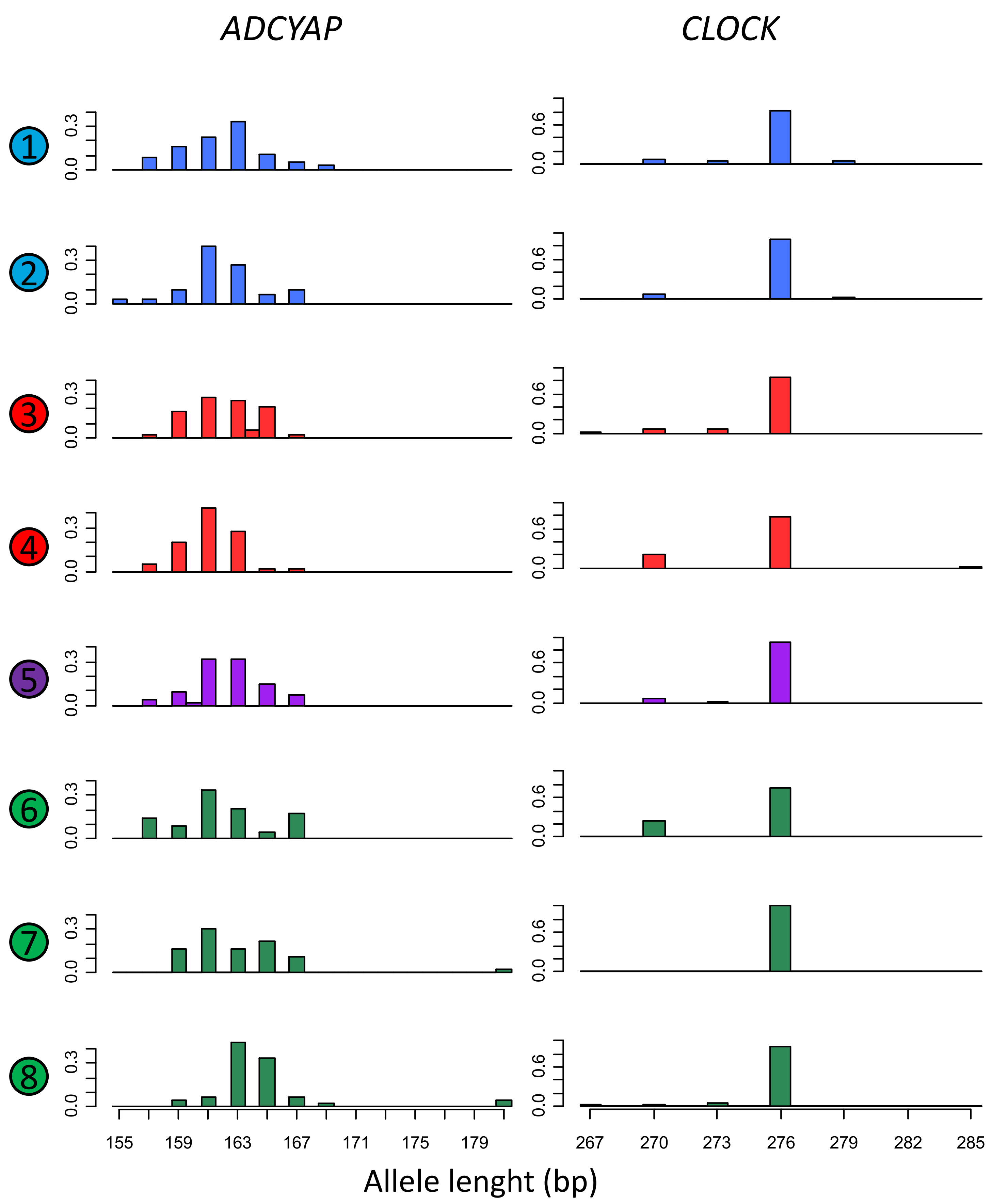


FIGURE 3: *ADCYAP* length and migratory restlessness

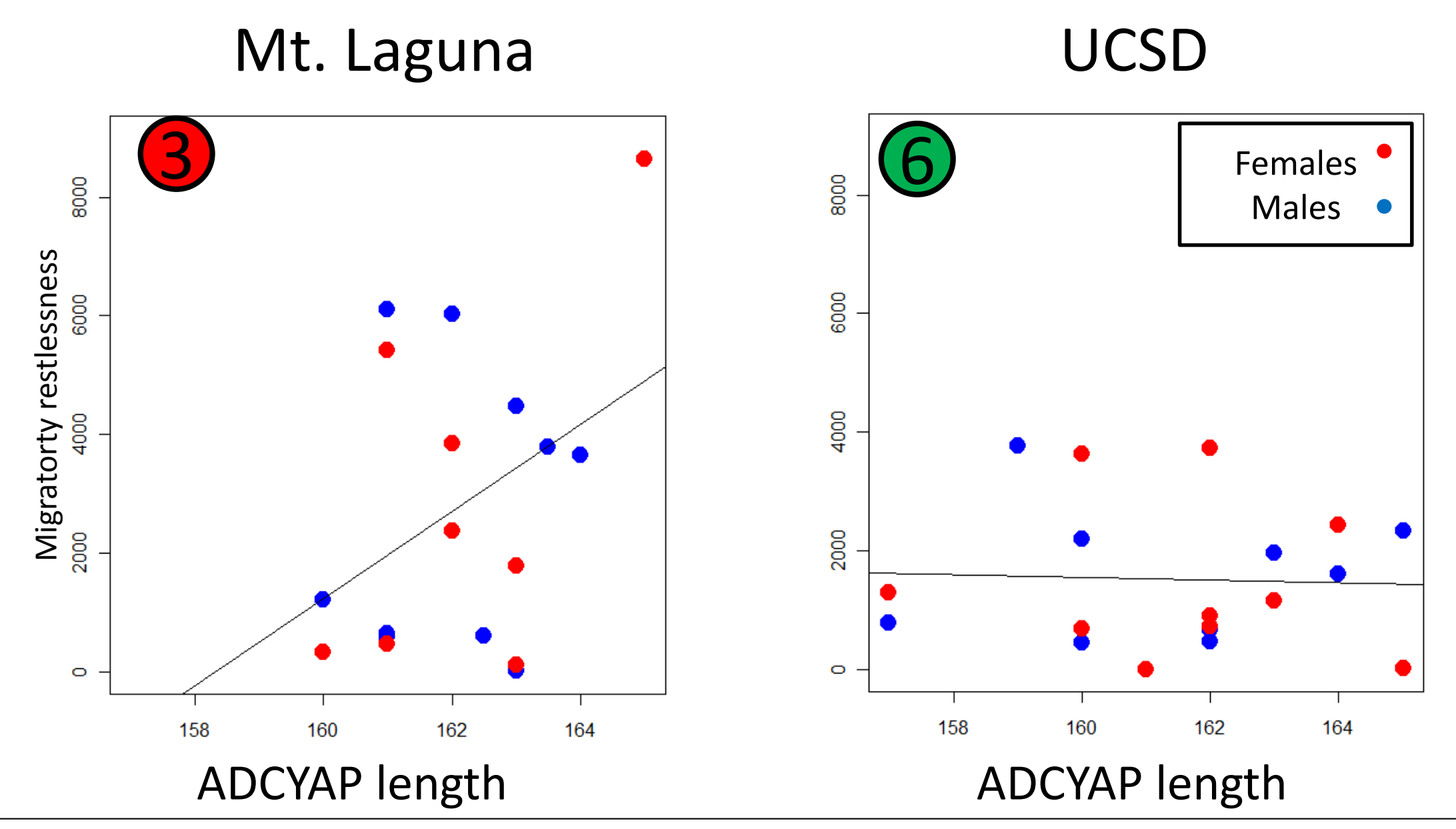
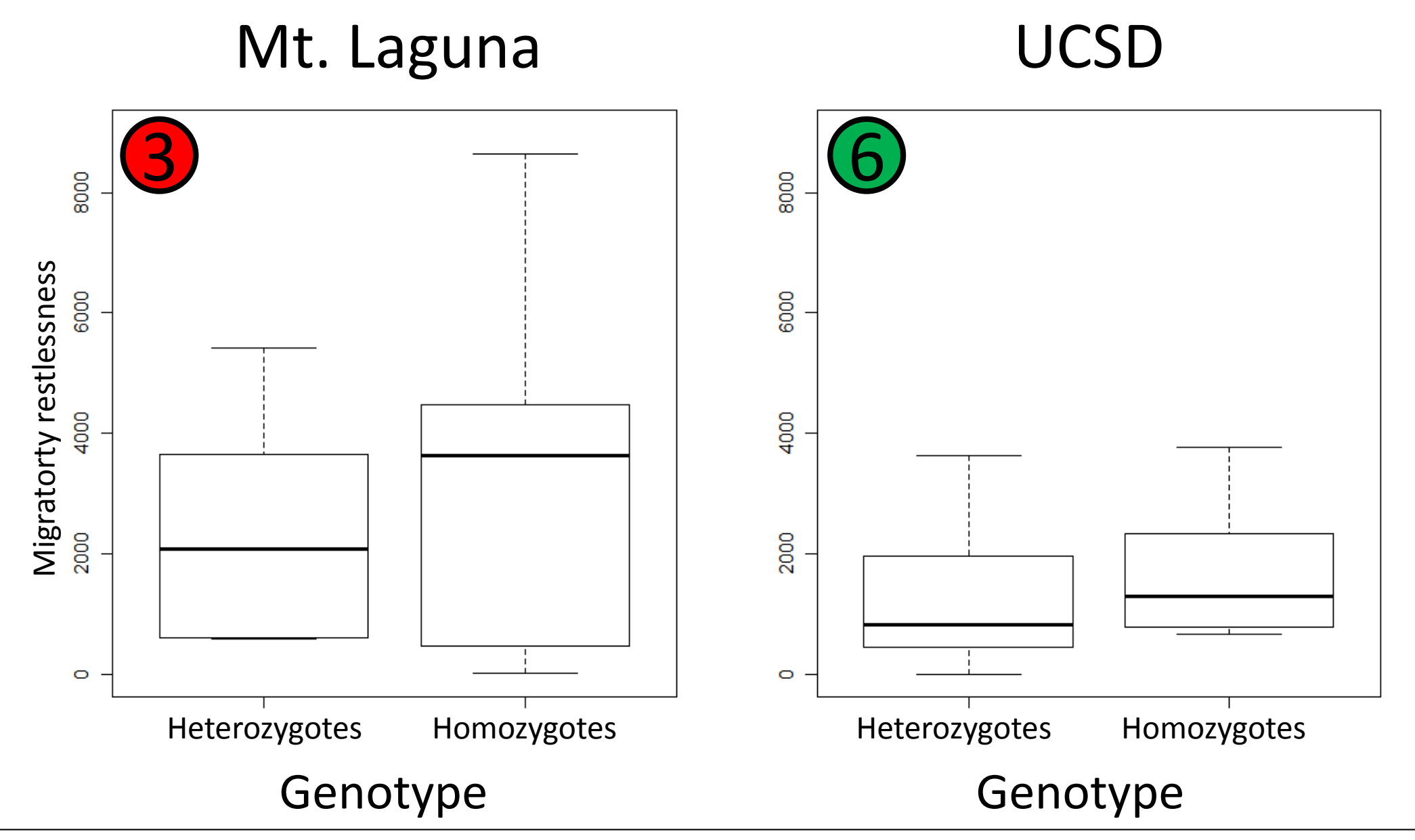


FIGURE 4: *CLOCK* genotype and migratory restlessness



RESULTS

ADCYAP

- All populations show TR variation in *ADCYAP* gene (Figure 2a).
- No consistent pattern of distribution of *ADCYAP* allele frequencies between populations with similar migratory phenotypes (Figure 2a).
- Mean individual allele length positively correlated ($r^2=0.15$, $p=0.11$) with migratory restlessness in the migratory population tested (Figure 3).

CLOCK

- Most population show TR variation, but one allele dominant in all populations (Figure 2b).
- No consistent difference in *CLOCK* allele frequencies between populations with different migratory phenotypes.
- Individuals heterozygous for short *CLOCK* allele tend to have lower migratory restlessness in both populations (Mt. Laguna $p=0.58$, UCSD $p=0.28$).

CONCLUSION

Variation in *ADCYAP* and *CLOCK* do not appear to explain population differences in migratory phenotype, but may contribute to population-specific individual variation in migratory restlessness.

FUTURE DIRECTIONS

- Test more individuals for migratory restlessness.
- Test more populations with different migratory phenotypes.
- Use our recently completed junco transcriptome[5] to search for other candidate genes.

FUNDING

- Funding for this project was provided by a NSF grant to EDK.

LITERATURE CITED

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ABSTRACT: Avian migration has fascinated behavioral biologists for generations, yet much remains to be learned about the genetic and neural bases for variation in its timing and occurrence. Greater insight into the molecular and neural mechanisms associated with migratory behavior will help to explain rapid evolution of migration and allow for more informed approaches to problems in conservation. A recent study by Mueller, Kempenaers and colleagues demonstrated that repeat length polymorphism in the gene *ADCYAP1* explains part of population variation in migratory behavior of the old-world warbler *Sylvia atricapilla*. To test the generality of this finding, we are investigating whether polymorphisms in *ADCYAP1*, as well as other candidate genes, help to explain variation in migratory behavior of the North American sparrow genus *Junco* (*J. hyemalis* & *J. phaeonotus*). Additional candidate genes were selected by surveying the recently completed *J. hyemalis* transcriptome for genes that contain repeat length polymorphisms and regulate key physiological or behavioral aspects associated with migration. We are examining DNA from 6 sub-species of *Junco* that are thought to have diverged from one another in the past 10,000 years. These subspecies represent a full spectrum of migratory variation, including sedentary populations and altitudinal, regional, and long-distance migrants. Our goals are to determine whether repeat length polymorphisms of candidate genes co-vary with migratory behavior of the different sub-species and to evaluate whether similar genetic mechanisms underlie behavioral diversity within different avian lineages.