

University of Montana

## ScholarWorks at University of Montana

---

Undergraduate Theses, Professional Papers, and Capstone Artifacts

---

2023

# A Comparison of Divergence in Non-Introgressing Genes Across a Chickadee Hybrid Zone

Eliana K. Lowe

University of Montana, Missoula, [el145276@umconnect.umt.edu](mailto:el145276@umconnect.umt.edu)

Follow this and additional works at: <https://scholarworks.umt.edu/utpp>



Part of the [Genomics Commons](#)

## Let us know how access to this document benefits you.

---

### Recommended Citation

Lowe, Eliana K., "A Comparison of Divergence in Non-Introgressing Genes Across a Chickadee Hybrid Zone" (2023). *Undergraduate Theses, Professional Papers, and Capstone Artifacts*. 444.

<https://scholarworks.umt.edu/utpp/444>

This Thesis is brought to you for free and open access by ScholarWorks at University of Montana. It has been accepted for inclusion in Undergraduate Theses, Professional Papers, and Capstone Artifacts by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact [scholarworks@mso.umt.edu](mailto:scholarworks@mso.umt.edu).

A COMPARISON OF DIVERGENCE IN NON-INTROGRESSING GENES ACROSS A  
CHICKADEE HYBRID ZONE

Eliana Lowe

Undergraduate thesis presented to faculty in partial fulfillment for High Honors in

Bachelor of Science  
Wildlife Biology  
Davidson Honors College

University of Montana  
Missoula, MT

May 2023

Dr. Zachary A. Cheviron

Dr. Jeffrey Good

Dr. Rena Schweizer

Dr. Gordon Luikart

## ABSTRACT

Hybridization between species plays a very important role in the overall evolutionary history of many taxa. The study of hybrid zones can also provide insight into how species are responding to environmental factors such as climate change and changes in species interactions. The hybrid zone between the northern black-capped chickadee (*Poecile atricapillus*) and the southern Carolina chickadee (*Poecile carolinensis*) provides an insight into how hybridization is affecting two distinct species. Black-capped and Carolina hybrids have reduced fitness in the wild. Hybrids tend to have higher basal metabolic rates, less muscle growth, and less neuron growth to prepare for winter caching. Locus-specific patterns of introgression can be used to identify loci that may contribute to species boundaries and fitness reduction in hybrids. Specifically, alleles that fail to introgress across hybrid zones could give an insight into why hybrids have reduced fitness. Our goal was to look at what functions those genes may have, and to see if the genes that don't introgress exhibit higher sequence divergence than those that do. We used mRNA transcript sequences generated from both black-capped and Carolina chickadees to determine whether loci that exhibit reduced introgression across the hybrid zone also show elevated sequence divergence compared to a sample of random genes, and whether these genes participate in key physiological processes. We calculated the mean percent sequence divergence for both the top ten non-introgressing genes and the ten randomly selected genes. The non-introgressing genes exhibited significantly higher sequence divergence than the random genes ( $p=0.0169$ ). This result demonstrates loci that fail to introgress have higher levels of sequence divergence than random genes. This suggests that there might be functional differences between species in the genes that could contribute to reduced fitness in hybrids.

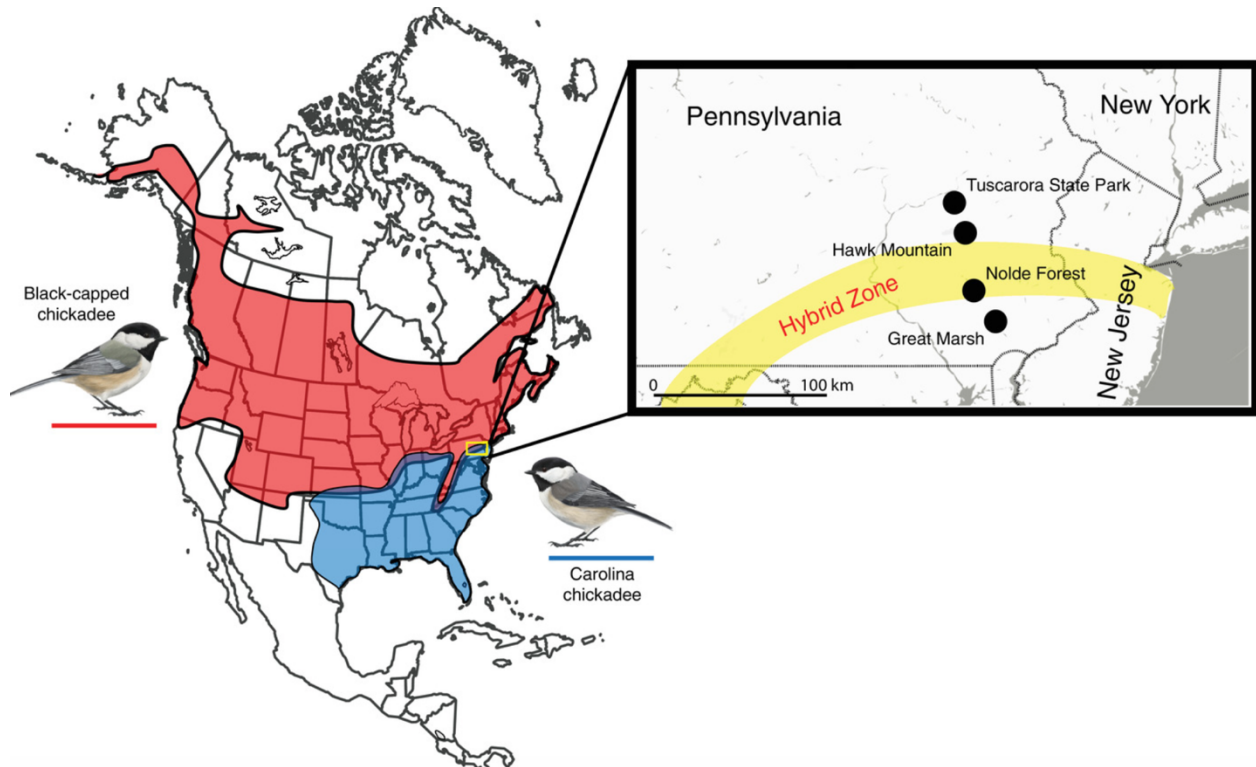
## INTRODUCTION

Interspecific hybridization is an important aspect of the evolution of many plant and animal groups, but the consequences and offspring of hybridization events can vary from being evolutionarily favored or being selected against in natural habitats (Abbott et al. 2013, Chunco 2014). If species are distantly related and have divergent genes because of their split, there is usually selection against hybridization where their hybrid offspring have reduced fitness. Speciation is on a spectrum, and complete reproductive isolation can take a long time to occur

between populations, especially if there is continued gene flow between two related species (Yamasaki et al. 2020). In taxa where there have been speciation events and then interspecific hybridization between the descendent species, non-introgressing genes tend to have high levels of divergence (Kulathinal et al. 2009, Minder 2008, Winger 2017, Yamasaki et al. 2020). Here, we look at two species of chickadee, the black-capped chickadee (*Poecile atricapillus*) and the Carolina chickadee (*Poecile carolinensis*), and their hybrid offspring to examine non-introgressing genes and their divergence between the two species.

Hybridization occurs regularly in the family *Paridae* between sister species, but black-capped and Carolina chickadee hybridization is unique in that the two species are not each other's closest relatives (Curry et al. 2007). After analysis of mtDNA, it was found likely that black-capped chickadees are more closely related to the high elevation mountain chickadees, and Carolina chickadees are more closely related to Mexican chickadees that reside in northern and central Mexico (Curry 2005). Black-capped chickadees are mainly a northern species, and the Carolina a southern species in North America. They hybridize at a specific point of range overlap between New Jersey and Kansas, then down into western North Carolina (Figure 1). In areas where the two species overlap, young can be sexually imprinted to another mating song by nestmates of a different species or by nearby adults (Grant and Grant 1997). Male black-capped chickadees have a characteristic *fee-bee* call, and male Carolina chickadees have a characteristic *see-bee-see-bay* call. The number of bilingual males increases in hybrid zones, which suggests that young chickadees in these areas can be influenced by other species and will sing both species songs during mating season (Curry et al. 2007). Hybridization can occur with mixed species pairs or in extra-pair copulations. In this case, hybrids mainly come from extra-pair copulation events (Curry et al. 2007). Black-capped females seem to prefer the more dominant male Carolinas and will seek them out in extra-pair copulation events while still caring for a nest with a black-capped male partner (Reudink et al. 2007).

FIGURE 1. Map of the black-capped chickadee and Carolina chickadee ranges, and their overlap. Inset is the hybrid zone located in New Jersey and Pennsylvania (Wagner et al. 2020).



Studies looking into black-capped and Carolina offspring have shown that hybrids tend to have lower winter survival rates than their parents (Olson et al. 2010, Reudink et al. 2007, Taylor et al. 2014). In black-capped chickadees and other cold-weather birds like juncos, cold temperatures trigger an upregulation of genes that are involved in angiogenesis, lipid transport, oxidative phosphorylation, hypertrophy, and neurogenesis (Cheviron and Swanson 2017, Stager et al. 2015). The seasonal transcriptomic responses of these genes correlate with the growth of pectoralis muscles, which are the main thermogenic organ in birds, and an increase in cellular metabolic activity (Cheviron and Swanson 2017). These physiological changes to cope with cold winter weather are not seen in hybrids, at least to the degree that is seen in black-capped chickadees (Olson et al. 2010, Wagner et al. 2020). Hybrids tend to have a lower thermogenic capacity, a higher basal metabolic rate, less muscle growth, and less neuron growth; all of which decrease the chances of survival in winter (Olson et al. 2010, Taylor et al. 2014a, Wagner et al. 2020). Hybrids also exhibit poor spatial memory compared to black-capped chickadees

(McQuillan et al. 2018, Wagner et al. 2020). Good spatial memory is needed in winter to remember where food caches are located.

The pressing issue of climate change makes study into the specifics of this hybrid zone paramount. Changing climates will most likely influence competitive interactions between species, and reproductive events between previously isolated populations can result in introgression and speciation, or extinction (Chunco 2014, Vallejo-Marín and Hiscock 2016). The hybrid zone is currently moving north about 1 kilometer a year since at least 2014, when the study was completed (Alexander et al. 2022, Taylor et al. 2014b). As the south grows too hot for Carolina chickadees, they push north into black-capped range. Black-capped chickadees are trapped, limited by climate and are unable to push any further north, or further down south into the Carolina chickadee range (Alexander et al. 2022, McQuillan et al. 2015, Taylor et al. 2014b). As climate change worsens, the black-capped habitat range will continue to shrink, and the probability of local extinction increases (Taylor et al. 2014b, Wagner et al. 2020). Black-capped chickadees have been projected to decline dramatically under all climate change scenarios in the Northeast, which is also where most hybridization events happen (Rodenhouse et al. 2008). This presents a complex problem where black-capped chickadees will not only be pressured by a loss of habitat, but also by the draw of black-capped females to the dominant Carolinas. Hybrid young take resources away from chicks that are pure-bred black-capped offspring, and that are more likely to survive the winter (Bronson et al. 2005). The parents raising low-fitness hybrids as the result of interspecific matings is a waste of their time, energy, and resources, as they are unlikely to survive the winter (Olson et al. 2010, Taylor et al. 2014b, Wagner et al. 2020).

Gene introgression occurs when hybrid offspring survive to reproduce to backcross into parent populations, and over time, this can result in the transfer of alleles from one species to another (Aguillon et al. 2022). Loci that are involved in reproductive isolation or hybrid fitness reductions have very low rates of introgression (Taylor et al. 2014a). Because hybrids exhibit reduced over-winter survival, alleles that don't introgress well across the hybrid zone may contribute to cold tolerance or other aspects of survival in harsh winter conditions.

## QUESTIONS AND HYPOTHESES

The primary goal of this study was to determine which genes introgress the least, and then examine the causes of this reduction in introgression. Our primary hypothesis is that non-introgressing genes are the most divergent or have the most nucleotide differences between the aligned genes, and that they contribute to functions that are relevant for overwinter survival because there is likely selection on black-capped chickadees to survive winter that contributes to gene divergence.

## ORIGIN OF CHICKADEE TRANSCRIPTS

Our collaborators sampled black-capped chickadees and Carolina Chickadees from allopatric populations outside of the hybrid zone to get allele frequencies that are distant from the contact zone. Allopatric populations were chosen to get representative black-capped and Carolina alleles that are distant from the contact zone. Black-capped chickadees were sampled in northern New York, and Carolina chickadees were sampled in Baton Rouge, Louisiana (n=5/species). Our collaborators generated *de novo* transcriptome assemblies from pectoralis muscle using standard approaches. Briefly, they isolated total RNA using DNeasy RNA extraction kits (Qiagen), and prepared sequencing libraries using Illumina TrueSeq kits. Individual RNA libraries were barcoded using unique indices and pooled for sequencing. The resultant sequencing data were *de novo* assembled using Trinity (Hass et al. 2014) and annotated using TransDecoder (<https://github.com/TransDecoder/TransDecoder>).

## METHODS

### *Non-Introgressing Genes*

We used an unpublished dataset of locus-specific introgression rates to choose a set of candidate loci for sequence analysis. These data were derived from whole genome sequences from a set of individuals sampled along two transects of the black-capped/Carolina chickadee hybrid zone (G. Semenov and S. Taylor unpubl. data). Cline widths for biallelic SNP were calculated using HZAR (Derryberry et al. 2014) SNPs were ranked by cline width, with narrow cline widths being indicative of reduced introgression rates. In cases where multiple SNPs within a gene

exhibit variable cline widths, we selected the single steepest SNP cline to represent the gene. We extracted transcript sequences for the top ten non-introgressing genes (those with the narrowest cline widths) from our de novo transcriptome assemblies. We also selected ten other randomly chosen genes to serve as a control, and extracted transcript sequences for these genes as well. The transcript sequences were numbered and selected using a random number generator. Sequences were BLASTed to get the gene name. We used GeneCards (Rebhan et al. 1997) to examine inferred function of the genes.

### *Gene Alignments*

We attempted to restrict our analysis to one-one orthologs. We took several precautions to avoid comparisons to paralogs. First, unannotated genes were excluded. Only genes that were annotated and represented in both species' assembled transcriptome were analyzed.

Extracted transcript sequences were aligned using Geneious v. 2023.0.4., and the percent sequence similarity was calculated for all 20 transcript alignments (10 candidate and 10 control genes). If the alignment generated was less than 85% similar, the gene was excluded as it may represent a paralogous alignment. In the alignments we made, we noticed that alignments were either above 85% or 50% or below, and so we chose the 85% similarity as the cut off for inclusion. In such cases with paralogous alignments, we moved on to the next gene in the cline list.

### *Data Analysis*

The mean percent similarity of the nucleotides between the top ten non-introgressing genes and the ten random genes was calculated using R Studio (R Core Team 2022). We ran a Shapiro-Wilks test to see if the percent similarities fit a normative curve. The resulting p-values ( $4.429e^{-06}$  for the top ten genes and  $2.333e^{-05}$  for the ten random genes) suggested that the data did not fit a normative curve, so we used a Wilcoxon ranked sum test to compare the two groups of percent similarity and see if the difference between the percentages was significant. We used R Studio (R Core Team 2022) to run all statistical tests.



## RESULTS

### *Gene Functions*

The top ten non-introgressing genes participated in functions that may improve winter survival such as fatty acid transport, oxidative phosphorylation, and learning and memory (Table 1). The ten random genes are mostly implicated in general cell regulation functions such as protein breakdown, cytoskeleton shape, and transcription (Table 2)

TABLE 1. List of the top ten non-introgressing genes and the inferred functions that each of them have (Rebhan et al. 1997).

<b>Gene Name</b>	<b>Function</b>
ABCA1	ATP-binding cassette transporters and cellular lipid removal.
COMMD10	Modulates activity of ubiquitination (protein homeostasis – removing damaged or unwanted proteins)
ISOC1	Located in the peroxisome, where oxidative reactions are carried out.
DGKQ	Regeneration of phosphatidylinositol in cell signal transduction.
AUH	Mitochondrial protein synthesis
SPTLC1	Sphingolipid biosynthesis (located in the brain, sphingolipids participate in tissue development, cell recognition, and adhesion)
SVEP1	Enables calcium ion binding for epidermis and lymph development
ALDH7A1	Detoxification of aldehydes from alcohol metabolism and lipid peroxidation
GAK	Control of phosphorylation
SHC3	Synaptic transmission and learning or memory

TABLE 2. List of the ten randomly selected genes and the inferred functions they have (Rebhan et al. 1997).

<b>Gene Name</b>	<b>Function</b>
SEMA3C	Neuronal guidance cues
ACACA	Catalyzation of acetyl-CoA in fatty acid synthesis
ARHGEF11	Implicated in many cellular processes initiated by extracellular stimuli
FGD6	Regulation of cell shape
ZFYVE6	Actin cytoskeleton and cell shape

SLC6A4	Transport of serotonin in synaptic spaces
ZFAND2B	Proteosome to degrade misfolded or toxic proteins
FOXP1	Gene transcription regulation
ZNF335	Enhances transcription activation and alters chromatin structure
FARS2	Transfers phenylalanine to tRNA and influences mitochondrial protein translation

---

### *Sequence Differences*

We found that the top ten non-introgressing genes tend to have more nucleotide differences than average than the ten random genes (p-value = 0.0169, Figure 2). This result suggests that loci that do not introgress across species boundaries are more divergent than random loci.



FIGURE 2. Bar graph of the percent similarity means and the standard error.

### **DISCUSSION**

In this study, we found that the top ten non-introgressing genes were more divergent than ten random genes and speculated that the inferred functions of the top ten non-introgressing genes could be involved with winter survival through cognitive and physiological functions. Even though we recognize black-capped and Carolina chickadees today as two distinct species, this doesn't mean that their divergence from each other has stopped. Speciation is still continuing in

these chickadees; complete reproductive isolation does not happen until the latest stage of speciation (Wagner et al. 2020, Yamasaki et al. 2020). The mechanisms behind complete reproductive isolation are mostly unknown, although we do know that a period of complete geographic isolation is one of the factors that is critical for the onset of reproductive isolation and can push isolation into its final stages (Yamasaki et al. 2020). The production of sterile male offspring can also be a sign of late-stage reproductive isolation (Nosil and Flaxman 2011, Yamasaki et al. 2020).

These chickadees are still overlapping in their ranges, and this overlap will most likely persist into the future as the hybrid zone moves north (Alexander et al. 2020, Reudink et al. 2007, Taylor et al. 2014b). Male hybrids that do survive the winter still retain the ability to have young as well, so these chickadees are probably not entering into late-stage reproductive isolation yet (Bronson et al. 2005, Curry et al. 2007). Even a small amount of gene flow can slow down complete reproductive isolation, so as long as these two species remain in contact, hybrid offspring will still be produced (Burney and Brumfield 2009, Claramunt et al. 2012, Gavrilets 2000). Sudden complete reproductive isolation in the presence of gene flow is rare, and unlikely to occur in most populations (Hendry et al. 2009, Yamasaki et al. 2020).

To sum up, reproductive isolation is on a continuum, and as species continue to diverge, the differences in genes can prevent the production of fit hybrid offspring due to low introgression of key genes. It has been shown that the most divergent genes are the least introgressed across species boundaries in many taxa such as sticklebacks, some plants, *Drosophila*, and Andean cloud forest birds (Kulathinal et al. 2009, Minder 2008, Winger 2017, Yamasaki et al. 2020). Our results do support this idea that genes that don't introgress are the most divergent in the genome.

Overall, these results are very preliminary. This thesis was meant to serve as a general look at the differences in genes between the two chickadee species, and to speculate on one aspect of non-introgression into hybrid offspring. It is essential to know if nonsynonymous divergence rate correlated with divergence, such as doing dN/dS calculations would give further insight into why these nucleotides are different between the species. Our results are constrained by only looking at ten genes; and that the transcripts only come from five individuals for each chickadee species.

This project could be expanded by looking at the full range of non-introgression genes and the difference between black-capped and Carolina transcripts. The fact that our p-value was significant indicates that giving the gene differences a further look is worth the time and effort. There could also be more future work into the genotype-to-phenotype relationship in non-introgressing genes, and to look more at what physiological changes and stressors are impacting the winter survival of parents and hybrids in natural environments.

We found a pattern that genes that have narrow cline widths tend to have higher overall divergence. This result needs more interpretation in order to fully realize what is causing these differences in sequence between the two species. One interpretation, and the one that we have relied on being true for this case, is that higher divergence at functionally important sites is due to positive natural selection in one or both species, and that this divergence leads to incompatibilities in hybrids. However, we cannot rule out that this pattern can also be the result of relaxed selection on functionally important sites. Parsing this variation into nonsynonymous and synonymous substitutions can give insight into differentiating these alternatives, but we just did not have the time to explore them.

These approaches can give us insight into the genes that are important for reduced hybrid fitness. There are a number of natural factors that may influence interbreeding between black-capped and Carolina chickadees, and many of these natural factors have a genetic basis, such as the ability to learn (McQuillan et al. 2018, Wagner et al. 2020). The mechanisms of divergence and how that leads to reduced introgression are still much of a mystery. We know that increased divergence most likely leads to non-introgression of those genes in hybrid offspring, but the continuum of speciation and eventual complete reproductive isolation from the first barriers of speciation needs to be studied more to understand how species might evolve in the future in changing landscapes and climates (Kulmuni et al. 2020, Nosil et al. 2017). There is most likely substantial selection against hybrids, and reproductive productivity is generally reduced in the hybrid zone for both black-capped and Carolina chickadees (Bronson et al. 2005, Wagner et al. 2020). In the case of these chickadees, these really divergent genes that don't introgress may contribute to reduced hybrid fitness and perhaps the eventual evolution of reproductive isolation (Taylor et al. 2014a, Wagner et al. 2020, Winger et al. 2017).

## LITERATURE CITED

- Abbott R., Albach D., Ansell S., Arntzen A. W., Baird S. J. E., Bierne N., Boughman J., Brelsford A., Buerkle C. A., Buggs R., Butlin R. K., et al. 2013. Hybridization and speciation. *Journal of Evolutionary Biology* **26**(2):229-246.
- Aguillon S. M., Dodge T. O., Preising G. A., Schumer M. 2022. Introgression. *Current Biology* **32**(16):R865-R868.
- Alexander A., Robbins M. B., Holmes J., Moyle R. G., Peterson A. T. 2022. Limited movement of an avian hybrid zone in relation to regional variation in magnitude of climate change. *Molecular Ecology* **31**(24):6634-6648.
- Bronson C. L., Grubb T. C. Jr., Sattler G. D., Braun M. J. 2005. Reproductive Success Across The Black-Capped Chickadee (*Poecile atricapillus*) and Carolina Chickadee (*P. carolinensis*) Hybrid Zone in Ohio. *The Auk* **122**(3):759-772.
- Burney C. and Brumfield R. 2009. Ecology predicts levels of genetic differentiation in Neotropical birds. *The American Naturalist* **174**:358-386.
- Cheviron Z. A. and Swanson D. L. 2017. Comparative Transcriptomics of Seasonal Phenotypic Flexibility in Two North American Songbirds. *Integrative and Comparative Biology* **57**(5):1040-1054.
- Chunco A. J. 2014. Hybridization in a warmer world. *Ecology and Evolution* **4**(10):2019-2031.
- Claramunt S., Derryberry E. P., Remsen J. V., Brumfield R. T. 2012. High dispersal ability inhibits speciation in a continental radiation of passerine birds. *Proceedings of the Royal Society B: Biological Sciences* **279**:1567-1574.
- Curry R. L. 2005. Hybridization in Chickadees: Much to Learn From Familiar Birds. *The Auk*

**122(3):747-758.**

- Curry R. L., Rossano L. M., Reudink M. W. 2007. Behavioral aspects of chickadee hybridization. *Ecology and Behavior of Chickadees and Titmice: An Integrated Approach* 95-110.
- Derryberry E. P., Derryberry G. E., Maley J. M., Brumfield R. T. 2014. HZAR: hybrid zone analysis using an R software package. *Molecular Ecology Resources* **14(3):652-663.**
- Gavrilets S. 2000. Waiting time to parapatric speciation. *Proceedings of the Royal Society B* **267:2483-2492.**
- Grant P. R. and Grant B. R. 1997. Hybridization, Sexual Imprinting, and Mate Choice. *The American Naturalist* **149(1):1-28.**
- Haas B. J., Papanicolaou A., Yassour M., Grabherr M., Blood P. D., Bowden J., Cougar M. B., Eccles D., Li B., Lieber M., MacManes M. D. et al. 2014. *De novo* transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. *Nature Protocols* **8(8).**
- Hendry A. P., Bolnick D. I., Berner D., Peichel C. L. 2009. Along the speciation continuum in sticklebacks. *Journal of Fish Biology* **75(8):2000-2036.**
- Kulathinal R. J., Stevison L. S., Noor M. A. F. 2009. The Genomics of Speciation in *Drosophila*: Diversity, Divergence, and Introgression Estimated Using Low-Coverage Genome Sequencing. *PLoS Genetics* **5(7):e1000550.**
- Kulmuni J., Butlin R. K., Lucek K., Savolainen V., Westram A. M. 2020. Towards the completion of speciation: the evolution of further reproductive isolation beyond the first barriers. *Philosophical Transactions of the Royal Society* **375:20190528.**

- McQuillan M. and Rice A. M. 2015. Differential effects of climate and species interactions on range limits at a hybrid zone: potential direct and indirect impacts of climate change. *Ecology and Evolution* **5**(21):5120-5137.
- McQuillan M. A., Roth II T. C., Huynh A. V., Rice A. M. 2018. Hybrid chickadees are deficient in learning and memory. *Evolution* **72**(5):1155-1164.
- Minder A. M., Widmer A. 2008. A population genomic analysis of species boundaries: neutral processes, adaptive divergence and introgression between two hybridizing plant species. *Molecular Ecology* **17**(6):1552-1563.
- Nolte A. W., Tautz D. 2010. Understanding the onset of hybrid speciation. *Trends in Genetics* **26**(2):54-58.
- Nosil P. and Flaxman S. M. 2011. Conditions for mutation-order speciation. *Proceedings of the Royal Society B: Biological Sciences* **278**:399-407.
- Nosil P., Feder J. L., Flaxman S. M., Gompert Z. 2017. Tipping points in the dynamics of speciation. *Nature Ecology and Evolution* **1**(1).
- Olson J. R., Cooper S. J., Swanson D. L., Braun M. J., Williams J. B. 2010. The Relationship of Metabolic Performance and Distribution in Black-Capped and Carolina Chickadees. *Physiological and Biochemical Zoology* **83**(2).
- Rebhan M., Chalifa-Caspi V., Prilusky J., Lancet D. 1997. GeneCards: integrating information about genes, proteins and diseases. *Trends in Genetics* **13**(4):163.
- Reudink M. W., Mech S. G., Mullen S. P., Curry R. L. 2007. Structure and Dynamics of the Hybrid Zone between Black-Capped Chickadee (*Poecile atricapillus*) and Carolina chickadee (*Poecile carolinensis*) in Southeastern Pennsylvania. *The Auk* **124**(2):463-478.

- R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rodenhouse N. L., Matthews S. N., McFarland K. P., Lambert J. D., Iverson L. R., Prasad A., Sillett T. S., Holmes T. R. 2007. Potential effects of climate change on birds of the Northeast. *Mitigation and Adaptation Strategies for Natural Change* **13**:517-540.
- Stager M., Swanson D. L., Cheviron Z. A. 2015. Regulatory mechanisms of metabolic flexibility in the dark-eyed junco (*Junco hyemalis*). *Journal of Experimental Biology* **218**(5):767-777.
- Taylor S. A., Curry R. L., White T. A., Feretti V., Lovette I. 2014. Spatiotemporally consistent genomic signatures of reproductive isolation in a moving hybrid zone. *Evolution* **68**(11):3066-3081.
- Taylor S. A., White T. A., Hochachka W. M., Ferreti V., Curry R. L., Lovette I. 2014. Climate-Mediated Movement of an Avian Hybrid Zone. *Current Biology* **24**:1-6.
- Vallejo-Marín M., Hiscock S. J. 2016. Hybridization and species speciation under global change. *New Phytologist* **211**(4):1170-1187.
- Wagner D. N., Curry R. L., Chen N., Lovette I. J., Taylor S. A. 2020. Genomic regions underlying metabolic and neuronal signaling pathways are temporally consistent in a moving avian hybrid zone. *Evolution* **74**(7):1498-1513.
- Winger B. M. 2017. Consequences of divergence and introgression for speciation in Andean cloud forest birds. *Evolution* **71**(7):1815-1831.
- Yamasaki Y. Y., Kakioka R., Takahashi H., Toyoda A., Nagano A. J., Machida Y., Møller P. R.,



Kitano J. 2020. Genome-wide patterns of divergence and introgression after secondary contact between *Pungitius* sticklebacks. *Philosophical Transactions of the Royal Society B* **375**:20190548