Mitochondria power the evolution of bird migration

Background: Migration is a global event that many species of birds take part in every year. Migration exists across numerous avian families and current evidence reveals that migration did not evolve during a singular event but rather, has been gained and lost numerous times via convergent evolution[1,2]. Studies have revealed that birds have higher maximum metabolic rate relative to other vertebrates [3], and that migrants display plastic changes in organ size [4,5], lipid deposits [6], and hematological traits that support migration [7,8]. Nevertheless, a critical component of migration that is vastly understudied are the mechanisms of energy production. Collectively the many physiological adaptations present in migratory birds are evidence of the process of symmorphosis, which predicts that the capacity of all parts of a physiological system must be matched with an overall functional demand [9]. **Symmorphosis is the basis for why I predict that physiological adaptations must occur within the mitochondria to meet the demands of migration.** Mitochondria are the primary source of adenosine triphosphate (ATP) production in the cell [10]. In the mitochondria, respiration occurs along the electron transport system through the process of oxidative phosphorylation (OXPHOS). There have been few studies on mitochondrial respiration in birds and even less on mitochondrial respiration in migrants [11–13]. **To provide insight into the evolutionary convergence in mitochondrial performance, I propose to determine which mitochondrial adaptations are common to avian migrants.**

For this investigation, I will collect birds and house them at Auburn's world-class aviaries in accordance with Auburn IACUC and under the guidance of Dr. Geoffrey Hill, Curator of Birds at Auburn University (AU). Both a migratory and non-migratory species was selected from five avian families (Parulidae, Cardinalidae, Troglodytidae, Turdidae, and Icteridae). The focal species were selected based on conservation status (only species of least concern will be used), feasibility, and capture rate near Auburn, Alabama. Twenty individuals of each species will be collected as our preliminary work has showed this to be sufficient to obtain statistical significance. All birds will be housed with artificial light to match photoperiods of both peak fall and spring migration in addition to mid-winter (or non-breeding) period in Alabama. Previous studies have shown that photoperiod stimulates migratory condition in birds and can be experimentally manipulated with success [14]. Pre-migratory condition will be determined by monitoring adipose fat, hematocrit levels, weight, and migratory restlessness (zugunruhe). Both migratory and non-migratory counterparts will be sacrificed within a five-day period of one another once pre-migratory condition is reached for the migratory groups. Both liver and pectoralis muscle tissue will be excised for mitochondrial testing.

Aim 1: Determine if mitochondrial adaptation plays a significant role in bird migration. H1: Migratory birds will have higher mitochondria volume, maximal respiration, and higher ATP production than closely related non-migratory species. Additionally, I expect to see higher efficiency within some of the individual electron transport system complexes. Mitochondria will be isolated via differential centrifugation in collaboration with Dr. Andreas Kavazis, School of Kinesiology at AU. Mitochondrial oxygen consumption, a proxy for energy output, will be measured polarographically in a respiration chamber maintained at 40°C (Hansatech Instruments, United Kingdom)[15]. Citrate-synthase activity assays will be conducted to determine mitochondrial volume. Markers for mitophagy and mitochondrial biogenesis will be analyzed using Western blots. I will quantify complex activity assays to test efficiency differences between the electron transport complexes (1-4) for the migratory and non-migratory species [16]. I will correct for relatedness by using phylogenetic correction within my analyses to identify differences with the migrants and non-migrants and to identify mitochondrial traits that are common to migrants. **Preliminary Findings:** I recently compared non-migratory Nuttall's White-crowned Sparrows (n=23) to migratory Gambel's White-crowned Sparrows (n=42) and found statistical differences between mitochondrial capacity measured via Respiratory Control Ratio (RCR). Migrants were found to have higher pectoralis mitochondrial capacity than non-migrants ($p < 0.0001$) for both complex I and II driven respiration (**Fig. 1**).

Aim 2: Test whether mitochondrial adaptation has occurred alongside tissue and whole-organism level adaptations. Is symmorphosis occurring?

H2: Mitochondrial respiration in birds is positively correlated with other key physiological traits. In other words, adaptations for migration have evolved on all levels (subcellular, tissue, and whole-body organism). I will use our mitochondria respiration data and test for correlations with other physiological traits, namely metabolic rate, organ size, hematocrit, hemoglobin, and lipid catabolism.

Feasibility: The species I have selected are common near AU. **Field sites and permits have already been approved.** I am collaborating with experts in the School of Kinesiology and Biological Sciences. I have the necessary skills and access to cutting-edge lab equipment and facilities for this project's success. Further, I have the independent research experience needed to analyze, manage the data, and publish the results from this investigation.

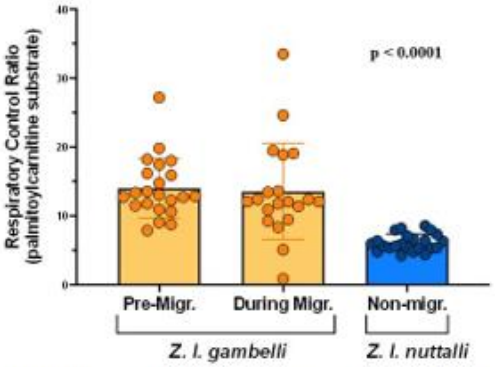

Figure 1: Mitochondrial respiratory capacity of Gambel's White-crowned Sparrow (*Zonotrichia leucophrys gambelli*) collected before and during migration and non-migratory Nuttall's White-crowned Sparrow (*Z.l.nuttalli*)

Intellectual Merit: Understanding how natural selection drives adaptations to meet energetically costly demands is crucial to understanding the mechanisms of trait evolution. **Mitochondrial adaptations and the role mitochondria may play in bird migration is not fully described or known.** Mitochondria may be a critical component to the evolution of bird migration that has gone mostly unnoticed in the evolutionary biology field. **Further, this study will look at where physiological traits have evolved through time to meet the demands of bird migration, a novel approach that can be applied to other migratory vertebrates and invertebrates.**

Broader Impacts: ***(1) Community Engagement and Education***. I will disseminate my findings to Alabama high schools in a format that is understandable to the lay person. **I am working with AU to provide undergraduates with hands-on field opportunities through my Undergraduate Bird Banding Training Program which I began in October 2020**. I teach participants skills that will help them to obtain wildlife and field-based jobs. ***(2) Management Implications.*** My research will help scientists better understand the physiological constraints of migration. Migratory bird populations are in decline [17]. Understanding species constraints will help researchers understand how migratory species may respond to climate change. I will collaborate with National Audubon to contribute my results to their Climate Initiative program to share how my findings relate to North American migratory species. Success of this study will be measured through presentations, publications, and number of people reached through my outreach initiatives.

References: **1.** Pulido (2007) *BioSci*. **2.** Zink (2011). *Bio. J. Lin. Soc*.**3.** Guglielmo et al. (2002) *Am. J. Physio.-Reg., Integr. and Compar. Physio.* **4.** Piersma et al. (1996) *Physio. Zoo*. **5.** Weber & Piersma (1996) *J. Avian Bio.* **6.** Jenni & Jenni-Eiermann (1998) *J. Avian Bio.***7.** Krause et al. (2016) *Physio. & Biochem. Zoo.* **8.** Yap et al. (2019). *Sci Rep*. **9.** Weibel et al. (1991) *Pro. Nat. Acad. Sci*. **10.** Gnaiger et al. (2018) *MitoFit Preprint Arch*. **11.** Kuzmiak et al. (2010) *The FASEB Journal*. **12.** Suarez et al. (1991) *Pro. Nat. Acad. Sci*. **13.** Toews et al. (2014) *Evolution***. 14.** Assadi & Fraser (2021) *Pro. R. Soc. B* **15.** Messer et al. (2004) *Am. Journal Physio.-Cell Physio.* **16.** Trounce et al. (1996) *Methods in enzymo*. **17.** Rosenberg et al. (2019) *Science*.