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RESEARCH ARTICLE

A migratory lifestyle is associated with shorter telomeres in a songbird (*Junco hyemalis*)

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ABSTRACT

For birds, a migratory lifestyle confers several benefits including avoidance of harsh winters and increased access to food resources during the breeding season. However, migration is energetically costly and elevates oxidative stress, which may contribute to increased mortality during migration. Oxidative stress is known to shorten telomeres, which are protective DNA regions on the ends of chromosomes. Thus, one consequence of migration may be accelerated telomere attrition. A migratory lifestyle may also increase telomere shortening via reduced investment into self-maintenance, as high mortality during migration may cause migrants to invest more in reproduction than residents. We therefore hypothesized that greater telomere attrition may reflect a long-term cost of a migratory life history strategy. We predicted that, among individuals of the same age, migrants would have shorter telomeres as compared to residents. We compared first-year individuals in an overwintering population of Slate-colored Dark-eyed Juncos (*Junco hyemalis*) that included both a migratory (*J. h. hyemalis*) and resident (*J. h. carolinensis*) subspecies in western Virginia. As predicted, first-year migrants had shorter telomeres than first-year residents. Since members of both subspecies experienced the same winter conditions and had not yet bred, differences in telomere lengths are likely due to migration-related costs as this was the only energetically expensive life history stage that differed between subspecies. Telomere length differences between subspecies could also be due to differences in initial telomere lengths or loss during growth, which could reflect relative investment into self-maintenance. These results are consistent with the idea that accelerated telomere shortening is a potential cost of a migratory life history strategy. This consequence could be the result of direct metabolic costs of migration and/or a life history strategy that places less emphasis on self-maintenance.

Keywords: junco, life history, migration, songbird, telomere

Un estilo de vida migratorio se asocia con telómeros más cortos en un ave canora (*Junco hyemalis*)

RESUMEN

Para las aves, un estilo de vida migratorio brinda varios beneficios incluyendo evitar los inviernos severos y aumentar el acceso a los recursos alimenticios durante la estación reproductiva. Sin embargo, la migración tiene un costo energético alto y aumenta el estrés oxidativo, lo que puede contribuir a una mayor mortalidad durante la migración. Se sabe que el estrés oxidativo acorta el largo de los telómeros, que son regiones protectoras del ADN en los extremos de los cromosomas. Por ende, una consecuencia de la migración puede ser el desgaste acelerado del telómero. El estilo de vida migratorio también puede aumentar el acortamiento del telómero mediante la reducción de la inversión en auto mantenimiento, ya que la alta mortalidad durante la migración puede hacer que los migrantes inviertan más en reproducción que los residentes. Por ende, nuestra hipótesis es que un mayor desgaste del telómero puede reflejar un costo de largo plazo de una estrategia de historia de vida migratoria. Nuestra predicción es que entre los individuos de la misma edad, los migrantes deberían tener telómeros más cortos comparados con los residentes. Comparamos individuos del primer año de vida de una población invernante de *Junco hyemalis* que incluyó dos subespecies, una migratoria (*J. h. hyemalis*) y otra residente (*J. h. carolinensis*), en el oeste de Virginia. Como predijimos, los migrantes del primer año presentaron telómeros más cortos que los residentes del primer año. Debido a que los miembros de ambas especies experimentaron las mismas condiciones invernales y no habían aún criado, las diferencias en los largos del telómero son probablemente debidas a costos relacionados con la migración, ya que esta fue la única etapa energéticamente costosa de la historia de vida que difirió entre las subespecies. Las diferencias en el largo del telómero entre las subespecies también podrían deberse a diferencias en el largo inicial del telómero o a pérdidas durante el crecimiento, lo que podría reflejar la inversión relativa en auto mantenimiento. Estos resultados son consistentes con la idea de que un acortamiento acelerado del telómero es un costo potencial de una estrategia de historia de vida migratoria. Esta consecuencia podría deberse a costos metabólicos directos de la migración y/o a una estrategia de historia de vida que le otorga menos énfasis al auto mantenimiento.

Palabras clave: ave canora, historia de vida, *Junco hyemalis*, migración, telómero

INTRODUCTION

Migration occurs in a wide array of taxa, and allows individuals to exploit seasonally varying food resources and weather conditions. For birds, vernal (spring) migration increases reproductive success by allowing individuals to take advantage of abundant food resources during the breeding season, while autumnal migration increases survival via avoidance of harsh winter conditions (Lack 1968). Long-distance migration comes at an energetic cost, however (Wikelski et al. 2003), and may increase an individual's exposure to oxidative stress (Costantini et al. 2007). In addition to the metabolic costs incurred during migration (e.g., sustained flight, refueling at stopovers), migrants also expend significant energy both preparing (e.g., pre-migratory fattening, muscle hypertrophy) and recovering from migration (Jenni and Jenni-Eiermann 1998). Oxidative stress exposure during migration may also be heightened due to increased immune challenges, as migrants encounter a wide diversity of pathogens during migratory transit (Møller and Erritzøe 2001). Together, these energetic costs may contribute to the increased mortality rates seen during migratory vs. sedentary life history stages (Sillett and Holmes 2002, Klaassen et al. 2014). While several studies have examined these short-term costs of migration, few have considered possible long-term consequences.

Telomere attrition may reflect long-term costs of migration. Telomeres, located at the ends of eukaryotic chromosomes, are highly conserved, non-coding repetitive DNA regions that protect coding sequences from loss during normal cell division (Blackburn 2005). In addition to cell division, telomeres can also shorten during oxidative stress exposure (von Zglinicki 2002). Once telomeres reach a critically short length, cells undergo senescence (Blackburn 2005). Because of this, telomeres are thought to act as sentinels of DNA damage, and thus are useful tools for determining an animal's "biological age" (Monaghan 2010). Indeed, recent ornithological studies have linked telomere lengths and their relative shortening rates with lifespan (Haussmann and Mauck 2008, Heidinger et al. 2012). Increased telomere attrition rates have also been linked with energetically expensive time periods or circumstances, such as breeding (Bauch et al. 2013, Sudyka et al. 2014), stress during post-natal development (Herborn et al. 2014), and decreased time at wintering grounds (Schultner et al. 2014). Thus, telomere attrition might reflect the physiological costs involved in shaping whether animals engage in migration, but it has yet to be tested whether telomere loss differs between migrants and residents. High telomere attrition rates in migratory individuals could also be linked to a decreased investment in self-maintenance. Because mortality rates are high during the migratory life history stage (Sillett and Holmes 2002, Klaassen et al. 2014), migrants may trade off self-maintenance in favor of reproduction (Alonso-Alvarez et al. 2006). Regardless of whether migrants experience increased telomere attrition due to migration-related metabolic costs or a decrease in self-maintenance, a migratory life history strategy may be linked to shorter telomeres.

We studied the Slate-colored Dark-eyed Junco (*Junco hyemalis*) to test whether a migratory life history strategy is associated with altered telomere dynamics. We measured telomere lengths in resident (*J. h. carolinensis*) and migrant (*J. h. hyemalis*) subspecies that share the same overwintering site. We predicted that, when controlling for age, individuals from the migrant subspecies would have shorter telomeres than individuals from the resident subspecies.

METHODS

Resident (*J. h. carolinensis*, $n = 21$) and migrant (*J. h. hyemalis*, $n = 11$) juncos were captured using baited Potter traps and mist nets at Mountain Lake Biological Station (37.22°N, 80.32°W) in western Virginia, March 12–19, 2015. This sampling period was ~4–6 wk before first-clutch initiation for residents and 2–4 wk before peak migratory departure for migrants, as migrants breed a few weeks later than residents (Bauer et al. 2016, Fudickar et al. 2016). Migrant males, migrant females, resident males, and resident females were identified via bill color, wing chord length, and plumage characteristics (Ketterson and Nolan 1976, Nolan et al. 2002). Because telomere length can vary with age (Monaghan 2010), we compared only first-year (hatch-year) birds, as first-year individuals can be reliably identified via plumage characteristics and iris color (Ketterson 1979).

Blood samples (~60 μL) were collected from the alar wing vein with heparinized microhematocrit capillary tubes. Blood samples were centrifuged, separated, and erythrocytes stored in Longmire's lysis buffer solution at 2°C. Avian erythrocytes are ideal for telomere measurement because they are a nucleated, highly mitotic tissue (Nussey et al. 2014). We extracted DNA by adding 100 μL of erythrocyte samples (in Longmire's lysis buffer solution) to 100 μL phosphate buffer solution, and then used Macherey-Nagel Nucleospin Blood Kits (Macherey-Nagel, Bethlehem, Pennsylvania, USA) according to the manufacturer's instructions. DNA purity and concentration was determined with a Nanodrop 8000 spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). All samples had 260/280 ratios above 1.7 and 260/320 ratios above 1.8.

Telomere lengths were then measured via quantitative PCR (qPCR) using an Mx3000P (Stratagene, San Diego, California, USA), according to the methods of Cawthon

(2002) as adapted for Dark-eyed Juncos. Resident and migrant samples were evenly distributed across qPCR plates while also keeping experimenters blind to migrant/resident status during analysis. Although interstitial telomeric repeats are included in qPCR measurements, they are unlikely to differ between these 2 subspecies of juncos that have only recently diverged and cannot be genetically differentiated using microsatellite techniques (Milá et al. 2007, Milá et al. 2016).

For our single copy control gene, we used glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). *GAPDH* was verified as a suitable control gene as a melt curve analysis demonstrated a single peak in the dissociation curve; melting temperature (T_m) = 82.0°C. Amplified PCR product was also run on a 2% agarose gel to verify the amplification of a single, expected product (98 bp). *GAPDH* primers were: Zebra Finch *forward GAPDH* (5′-AACCAGCCAAGTACGATGACAT-3′) and Zebra Finch *reverse GAPDH* (5′-CCATCAGCAGCAGCCTTCA-3′). Telomere primers were: *forward tel1b* (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′) and *reverse tel2b* (5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′).

Each reaction (total volume of 25 μL) contained 10 ng DNA, 12.5 μL perfeCTa SYBR green supermix Low ROX (Stratagene), and 200 nM/200 nM *forward GAPDH*/*reverse GAPDH* or 200 nM/200 nM *forward tel1b*/*reverse tel2b*. Samples were run in triplicate, and *GAPDH* and telomere reactions were run on separate plates. For a standard curve, each plate contained a serial dilution (40, 20, 10, 5, and 2.5 ng) of the same reference sample of a single junco. All samples fell on the standard curve. qPCR reaction conditions for *GAPDH* were 10 min at 95°C, followed by 40 cycles of 30 s at 95°C and 30 s at 60°C, finishing with 1 min at 95°C, 30 s at 55°C, and 30 s at 95°C. qPCR reaction conditions for telomeres were 10 min at 95°C, followed by 27 cycles of 15 s at 95°C, 30 s at 58°C, and 30 s at 72°C, finishing with 1 min at 95°C, 30 s at 58°C, and 30 s at 95°C.

The number of PCR cycles (C_t) to reach a threshold (set by the 10 ng dilution of the reference sample) was measured for each sample. Telomere lengths were then quantified as T/S ratios (the ratio of telomere repeats (TTAGGG) to the number of copies of a "control" gene (*GAPDH*: Glyceraldehyde-3-phosphate dehydrogenase)) using the following formula: $2^{\Delta\Delta Ct}$, where $\Delta\Delta C_t = (C_t^{\text{telomere}} - C_t^{\text{GAPDH}})$ reference $- (C_t^{\text{telomere}} - C_t^{\text{GAPDH}})$ focal (Stratagene 2007). Intra-assay variation for *GAPDH* and telomere reactions was 0.25% and 0.77%, respectively. Inter-assay variation for *GADPH* and telomere reactions was 0.11% and 4.61%, respectively. Inter-plate variation for change in C_t values was 7.34%. Inter-assay variations were calculated using all triplicates. Inter-plate variations were calculated using the 10 ng dilution of the reference sample.

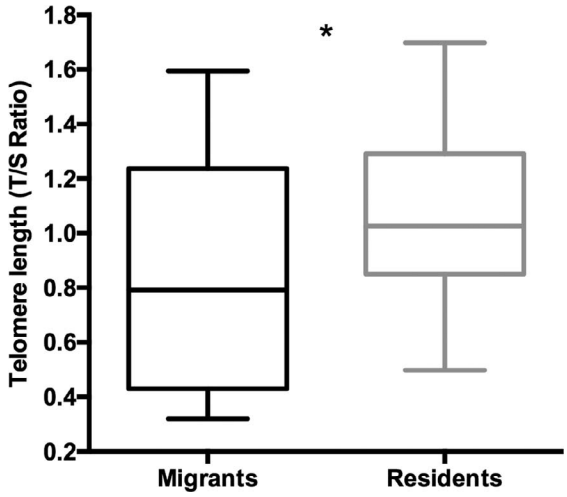

FIGURE 1. Migrants (*J. h. hyemalis*, $n = 11$) had significantly shorter telomeres (T/S ratio) than residents (*J. h. carolinensis*, $n = 21$) among first-year juncos sampled at their shared overwintering site. T/S ratios represent the ratio of (T): telomere repeats (TTAGGG) to (S): the number of copies of a "control" gene (*GAPDH*). The horizontal line represents the mean, the box represents one standard deviation above and below the mean, and whiskers represent the maximum and minimum T/S ratios.

Average efficiencies for *GAPDH* and telomere plates were 94.84% ± 2.28 and 87.58% ± 1.55 (mean ± SE), respectively. Although average efficiencies differed by 7%, the ranges overlapped and in all cases the efficiencies were within the recommended 85–115%. As samples were randomly distributed among plates, this slight difference in efficiency did not have any systematic effects on our findings.

Statistical Analyses

Telomere lengths were analyzed using a two-way ANOVA and met all normality and homogeneity assumptions. Independent variables included subspecies (migrant or resident), sex, and a subspecies by sex interaction. Results were considered significant if $p \leq 0.05$. Partial η^2 is reported for all significant effects. Partial η^2 values of 0.01, 0.09, and 0.25 are considered to represent small, medium, and large magnitudes of effect, respectively (Cohen 1988). All analyses were performed in SPSS*20 (IBM, Armonk, New York, USA).

RESULTS

Migrants had significantly shorter telomeres compared with residents (Figure 1; $F_{1,28} = 4.323$, $p = 0.047$, partial $\eta^2 = 0.134$). While there was a trend for males to have shorter telomeres than females ($F_{1,28} = 3.809$, $p = 0.061$, partial $\eta^2 = 0.120$), we found no significant interaction between subspecies and sex ($F_{1,28} = 0.255$, $p = 0.618$, partial $\eta^2 = 0.009$).

DISCUSSION

The purpose of this study was to determine whether migrant birds have shorter telomeres compared to resident birds following a single migration event. Consistent with this hypothesis, we found that telomeres were significantly shorter in first-year migratory juncos (*J. h. hyemalis*) compared to first-year resident juncos (*J. h. carolinensis*). Since short telomeres have been linked with increased senescence rates (Haussmann and Mauck 2008, Heidinger et al. 2012), our findings suggest that one long-term cost of a migratory lifestyle could be a reduced lifespan. This fits well with a previous study in migratory juncos that indicated mortality during migration increases with flight distance (Ketterson and Nolan 1982). For migrants, longevity costs due to migration are likely balanced by increased reproductive success at high-latitude breeding grounds where resources are abundant during chick-rearing (Lack 1968).

Shorter telomeres in our migrant individuals may directly reflect the high metabolic costs of migrating, as high metabolic rates increase oxidative stress exposure, which can accelerate telomere attrition (von Zglinicki 2002). If telomere loss can be solely attributed to the act of migration, then we would assume that our migrant and resident birds had similar telomere lengths at the beginning of life, which is likely as low haplotype and nucleotide diversity within the Dark-eyed Junco complex suggest these subspecies only very recently diverged (Milá et al. 2007, Milá et al. 2016). If this is the case, then the significant difference we saw in telomere lengths between migrants and residents is especially notable because migrants had completed only one migratory journey (from breeding to wintering grounds) before our sampling period in March. Given that both subspecies had not yet bred and shared the same overwintering conditions, the likelihood is enhanced that telomere differences are attributable to migration, rather than other energetically expensive life history stages such as breeding.

A non–mutually exclusive alternative is that shorter telomeres observed in migrants reflect a lower investment in self-maintenance. Because mortality risk is significantly higher during migratory than stationary periods (Sillett and Holmes 2002, Klaassen et al. 2014), selection may have shaped a strategy whereby migrants benefit from a life history strategy that places less value on self-maintenance and more on growth and reproduction. Low investment in telomerase and other cellular repair mechanisms could cause migrants to have shorter telomeres than residents upon fledging, as telomere attrition rates are highest early in life (Hall et al. 2004, Heidinger et al. 2012). Low repair rates would also cause increased telomere attrition during adulthood, as well. However, by migrating to high latitudes, the cost of low self-maintenance may be offset by increased food availability (Lack 1968), which could allow for higher hatching and fledging success rates..

Conclusions

This study is consistent with the hypothesis that a migratory life history strategy accelerates telomere loss in a small-bodied passerine. Future studies are needed to determine whether shorter telomeres in migrants reflect the metabolic costs of migration or are a result of a life history strategy that places less emphasis on self-maintenance. To do this, telomere lengths could be measured in individuals immediately before and after migration, and shortening rates then compared with other life history stages or with a resident subspecies over a similar timeframe. Additionally, future studies could also assess whether telomere lengths are negatively correlated with migration distance, as both feather hydrogen isotopes and the degree of Zugunruhe provide good estimates of breeding latitude (Berthold 1973, Hobson and Wassenaar 1997)

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Ethics statement: All procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee. Samples were collected under both federal (Bird Banding Permit #23867) and state (VA permit #047553) permission.

Author contributions: C.M.B., B.J.H., and T.J.G. conceived and designed the study. C.M.B. and T.J.G. collected the data. C.M.B. and B.J.H. analyzed the data. C.M.B., B.J.H., E.D.K., and T.J.G. all contributed to writing the manuscript.

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