

Electronic Thesis and Dissertation Repository

5-4-2020 2:00 PM

Correlations in movement behaviour over large and small geographic scales in song sparrows (*Melospiza melodia*)

Christopher D. Posliff, *The University of Western Ontario*

Supervisor: MacDougall-Shackleton, Elizabeth A., *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

© Christopher D. Posliff 2020

Follow this and additional works at: <https://ir.lib.uwo.ca/etd>



Part of the [Biology Commons](#)

Recommended Citation

Posliff, Christopher D., "Correlations in movement behaviour over large and small geographic scales in song sparrows (*Melospiza melodia*)" (2020). *Electronic Thesis and Dissertation Repository*. 7027. <https://ir.lib.uwo.ca/etd/7027>

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlsadmin@uwo.ca.

Abstract

In many animals, individuals show consistent variation in activity and movement, suggesting the existence of movement-related behavioural syndromes. I assessed the relationship between exploratory behaviour (over a small spatial scale) and migration distance (over a much larger scale) in song sparrows (*Melospiza melodia*). I quantified exploration using a novel environment test and inferred migration distance (overwintering latitude) using stable hydrogen isotope analysis of winter-grown claw tissue. Exploration was positively related to migration distance. I also investigated candidate mechanisms that if common to both exploration and migration, could explain the correlation between these behaviours. Circulating androgen levels were not associated with either movement behaviour, but at the dopamine receptor gene DRD4 one sequence variant was linked to exploration and another to migration distance. The observed relationship between small- and large-scale movement suggests the existence of a movement syndrome and provides insight into the evolution of movements over multiple geographic scales.

Keywords

Exploratory behaviour, migration distance, behavioural syndrome, movement behaviour, DRD4, androgens, stable hydrogen isotope, *Melospiza melodia*, novel environment test

Summary for Lay Audience

Individuals in many animal species show correlated differences in their behaviours and movement, suggesting the existence of behavioural syndromes. Behavioural syndromes are collections of multiple behaviours that show consistencies in different contexts. For example, individuals that are more aggressive than others in certain situations may also be bolder than their counterparts in other circumstances. Accordingly, behaviours related to movement on multiple scales, such as exploration over short distances and migration over longer distances, may show a relationship with each other. I tested the relationship between exploration and migration distance in a breeding population of song sparrows (*Melospiza melodia*). I placed individuals into a room with features they would not experience in their natural habitats and measured how much they explored this new environment. To quantify migration distance, I analyzed isotope composition of winter-grown claw tissue. The relative proportions of “heavy” and “light” forms of hydrogen in claw tissue reflect those of the area where the tissue was grown. Because these proportions vary predictably with latitude, stable isotope analysis of winter-grown tissue allows estimating how far south each bird migrated. Exploration levels were strongly correlated with latitudinal and total migration distance—more exploratory birds overwintered further south and thus migrated longer distances.

Exploration and migration might be correlated because they are both influenced by the same mechanism, for example the same genetic variant or hormone. I tested two candidate mechanisms that might link exploration to migration: androgen levels and variation at a dopamine gene. Androgen levels were not associated with either small-scale (exploration) or large-scale (migration distance) movement behaviours. However, variation at the dopamine receptor DRD4 was related to both exploratory behaviour and total migration distance. This gene is involved in the motivational and reward pathways. The relationship between small-scale movement (exploration) and larger-scale movement (migration) suggests movement may be a consistent trait, and part of a behavioural or ‘movement’ syndrome. This result improves our understanding of the evolution of movements over multiple geographic scales.

Acknowledgements

First of all, I need to thank my amazing supervisor, Professor Beth MacDougall-Shackleton, for all of her guidance and for being so encouraging and supportive throughout my Master's. Your enthusiasm and excitement for research has been so motivational, and without you I would most certainly not be able to complete this project. I must also thank my advisors, Professor Keith Hobson and Professor David Sherry, for all of their insight and guidance throughout the entirety of my research. The support and knowledge they contributed helped tremendously to shape this project into what it is today. Thank you to the members of the Mac-Shack lab (past, present, and honorary), including Tosha Kelly, Leanne Grieves, Lydia Balogh, Rachel Boyd, Melanie Denomme, Rebecca Howe, Haylee Begin-Dyck, Leticia Soares, and Rick Simpson, who all contributed so much to my Master's journey, from lab training, to advice on presentations, to all the support they gave me along the way. I need to thank Garth Casbourn and Professor Scott MacDougall-Shackleton for all of their help and support both in the field and in the lab, and for helping to build an exploratorium that would not blow over for a third time. Thank you to Jackson Kusack aiding greatly in my understanding and creation of the isoscape analyses. I am so grateful to the staff at the Queen's University Biological Station for providing an excellent environment for research and the Advanced Facility for Avian Research – Laboratory for Stable Isotope Science for their help with the stable isotope analysis. Thank you to all of my incredible friends, specifically Jordan Van Brenk, Emily Clayton, Brad Bork, Carlie Muir, Bonnie Alberry, Kaelyn Bumelis, Corrine Genier, and Céline Jennings, for their incredible help and support with the completion of my thesis. Finally, I would like to thank my parents, Dave and Irene Posliff, and my brother Dan Posliff, for being the most amazing and supportive family I could possibly ask for. This thesis would not be possible without you all, thank you so very much.

Table of Contents

Abstract.....	ii
Keywords	ii
Summary for Lay Audience.....	iii
Acknowledgements	iv
List of Tables	viii
List of Figures.....	ix
List of Appendices.....	xi
List of Abbreviations	xiii
1. Introduction.....	1
1.1 Behavioural syndromes	1
1.1.1 Movement syndromes.....	3
1.2 Migratory behaviour	3
1.3 Exploratory behaviour	5
1.4 Proximate causes of variation in movement behaviour.....	8
1.4.1 Androgens.....	8
1.4.2 Dopamine receptors	10
1.5 Study system.....	12
1.6 Objectives and hypothesis	13
2. Methods	18
2.1 Study animals and field methods	18
2.1.1 Novel environment test.....	19

2.2	Stable isotope analysis	23
2.2.1	Estimating total migration distance with isoscape assignment.....	25
2.3	Androgen assay.....	27
2.4	Genetic analysis of DRD4 exon 3	28
2.4.1	DRD4 exon 3 amplification.....	28
2.4.2	Sequencing.....	30
2.4.3	Surveying for SNPs	30
2.5	Statistical analysis.....	31
3.	Results.....	35
3.1	Variation in exploration	35
3.2	Variation in migration distance	40
3.3	Relationship between exploration and migration	46
3.4	Plasma androgen concentrations and movement behaviours	49
3.4.1	Androgens and exploration.....	49
3.4.2	Androgens and migration distance	55
3.5	DRD4 variation and association with movement behaviours.....	60
3.5.1	Genetic variation at DRD4 exon 3.....	60
3.5.2	DRD4 variation and movement behaviours	64
4.	Discussion	75
4.1	Overview.....	75
4.2	Relationship between exploration and migration distance	78
4.2.1	Exploration, migration, and date of first capture	80

4.3	Relationship between movement and circulating androgens.....	81
4.4	Sequence variation at DRD4 exon 3 associated with movement behaviours.....	83
4.4.1	Comparing DRD4 exon 3 variation between species	83
4.4.2	Movement behaviours and DRD4 exon 3 polymorphisms.....	84
4.5	Future studies.....	87
4.6	Conclusions.....	88
5.	References.....	90
6.	Appendix A.....	103
7.	Appendix B.....	111
8.	Curriculum Vitae.....	114

List of Tables

Table 3.1. Spearman correlation matrix of exploratory behaviours for all trials.....	36
Table 3.2. Spearman correlation matrix of exploratory behaviours for first ever trials. ..	37
Table 3.3. Loading scores for principal component analysis (PCA) of exploratory behaviours for all trials.	38
Table 3.4. Loading scores for principal component analysis (PCA) of exploratory behaviours for first ever trials.	39
Table 3.5. Repeatability of exploratory scores (PC1) between first and second trials.	41
Table 3.6. Linear regression model of latitudinal migration distance and exploratory scores.....	50
Table 3.7. Linear regression models of total migration distance including and excluding three northernmost values and exploratory scores.....	51
Table 3.8. Linear regression model of exploration scores (PC1) and logarithmically transformed androgen concentrations.	54
Table 3.9. Linear regression models of latitudinal migration distance or total migration distance including or excluding the three northernmost values and logarithmically transformed androgen concentration.....	58
Table 3.10. Single nucleotide polymorphisms (SNPs) identified in exon 3 of DRD4.	63
Table 3.11. Single nucleotide polymorphisms (SNPs) in exon 3 of the DRD4 gene, analyzed in association with movement behaviours.	65

List of Figures

Figure 1.1. Proximate mechanisms hypothesized to mediate the potential relationship between exploratory behaviour and migration distance.	16
Figure 2.1 Top down view of the novel environment room used to test exploratory behaviour.....	21
Figure 3.1. Likely overwintering origins of song sparrows based on $\delta^2\text{H}$ stable isotope values of claw samples.....	42
Figure 3.2. Correlation between latitudinal migration distance and total migration distance.	44
Figure 3.3. Correlation between exploration scores and latitudinal migration distance or total migration distance.....	47
Figure 3.4. Linear regression of first day of capture as a function of latitudinal migration distance or total migration distance.	52
Figure 3.5. Linear regression of movement behaviours as a function of the date of blood sampling.....	56
Figure 3.6. Great Tit and Song Sparrow protein alignment.....	61
Figure 3.7. Occurrence of C734T within exon 3 of the DRD4 gene in relation to exploration score and latitudinal migration distance or total migration distance.	66
Figure 3.8. Occurrence of A742G within exon 3 of the DRD4 gene in relation to exploration score and latitudinal migration distance or total migration distance.	68
.....	69
Figure 3.9. Occurrence of C827T within exon 3 of the DRD4 gene in relation to exploration score and latitudinal migration distance or total migration distance.	70

Figure 3.10. Occurrence of any SNP within exon 3 of the DRD4 gene in relation to exploration score and latitudinal migration distance or total migration distance. 73

Figure 4.1 Resulting relationships between proximate mechanisms hypothesized to mediate the relationship between exploratory behaviour and migration distance. 76

List of Appendices

Table A.1 AICc-ranked selection table for models predicting latitudinal migration distance (inferred from $\delta^2\text{H}$) as a function of exploratory scores and other potential covariates.	103
Table A.2 AICc-ranked selection table for models predicting total migration distance using the full dataset as a function of exploratory scores and other potential covariates.	104
Table A.3 AICc-ranked selection table for models predicting total migration distance excluding the three northernmost values as a function of exploratory scores and other potential covariates.	105
Table A.4 AICc-ranked selection table for models predicting exploration scores as a function of logarithmically transformed androgen concentration and other potential covariates.	106
Table A.5 AICc-ranked selection table for models predicting exploration scores as a function of logarithmically transformed androgen concentration and other potential covariates, for the subset of individuals that had blood collected and behavioural testing done within five days of one another.	107
Table A.6 AICc-ranked selection table for models predicting latitudinal migration distance (inferred from $\delta^2\text{H}$) as a function of logarithmically transformed androgen concentration and other potential covariates.....	108
Table A.7 AICc-ranked selection table for models predicting total migration distance using the full dataset as a function of logarithmically transformed androgen concentration and other potential covariates	109

Table A.8 AICc-ranked selection table for models predicting total migration distance excluding the three northernmost values as a function of logarithmically transformed androgen concentration and other potential covariates..... 110

List of Abbreviations

$\delta^2\text{H}$ – Stable Hydrogen Isotope Deuterium

$\delta^2\text{H}_c$ – Stable Hydrogen Isotope Deuterium derived from Claw samples

$\delta^2\text{H}_f$ – Stable Hydrogen Isotope Deuterium derived from Feather samples

AICc – Akaike Information Criterion corrected for small sample size

ANOVA – Analysis of Variance

AP – Alkaline Phosphatase

BLAST – Basic Local Alignment Search Tool

bp – Base Pair

CBS – Caribou Hoof Standard

CI – Confidence interval

df – Degrees of Freedom

DNA – Deoxyribonucleic Acid

dNTP – Deoxyribonucleotide Triphosphate

DRD2 – Dopamine Receptor Domain 2

DRD4 – Dopamine Receptor Domain 4

EDTA – Ethylenediaminetetraacetic Acid

GnRH – Gonadotropin-Releasing Hormone

HCl – Hydrochloric Acid

KCl – Potassium Chloride

KHS – Kudu Horn Standard

mRNA – Messenger Ribonucleic Acid

NCBI – National Center for Biotechnology Information

PC1 – Principal Component 1

PCA – Principal Component Analysis

PCR – Polymerase Chain Reaction

SNP – Single Nucleotide Polymorphism

TBE – Tris/Borate/EDTA

TE – Tris/EDTA

VSMOW-SLAP – Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation

1. Introduction

1.1 Behavioural syndromes

Like many traits, animal behaviours do not necessarily evolve independently but can instead evolve in concert with a suite of other (behavioural) traits. Correlations between behaviours may arise because both behaviours are influenced by a common underlying mechanism, and/or because both are subject to similar selective pressures (Moretz et al., 2007). Behavioural syndromes (Sih et al., 2004a; Sih et al., 2004b; Dingemanse et al., 2012) occur when behaviours are correlated across multiple timepoints or across different contexts, and have been found to exist in a wide array of taxa including fish (e.g. Bell, 2005; Martins & Bhat, 2014), birds (e.g. Myers & Hyman, 2016), mammals (e.g. Hertel et al., 2019), as well as in invertebrate species (Karlsson Green et al., 2016). For example, some individuals may be consistently bolder, shyer, or more exploratory than others in the same population. Similarly, individuals that are relatively bold in approaching predators may also take more risks in territorial interactions with conspecifics. Because they affect whether two or more behaviours evolve independently or in concert, behavioural syndromes can have important evolutionary consequences.

The lack of flexibility associated with behavioural syndromes may appear to be costly. Despite their constraints, however, behavioural syndromes can have important advantages relative to the alternative of complete behavioural flexibility (that is, of each behaviour evolving independently). Behaviours that are favoured in one particular environment or context may also be adaptive in other contexts an individual is likely to encounter, which could favour a more predictable behavioural suite. On the other hand,

behavioural syndromes can generate costly trade-offs when the optimal behaviour in one environment or context is very different from that in another, potentially having an effect on fitness (Evans et al., 2010; Sih & Del Giudice, 2012). For example, streamside salamander larvae (*Ambystoma barbouri*) show correlated foraging behaviours at night and during the daytime, even though the risk of predation is often higher during the day (Sih et al., 2003). The existence of limited plasticity and behavioural correlations can cause individuals to often exhibit behaviours that are suboptimal when viewed in isolation (Sih et al., 2004a), but correlational selection may shape the behaviours of animals, and cause adaptations for behaviours that are optimal to maximize fitness in their environment (Karlsson Green et al., 2016). Thus, there are costs and benefits to both behavioural syndromes and the alternative of behavioural flexibility.

Where behavioural syndromes are present, each individual has a particular combination of behaviours (behavioural type), but the behavioural syndrome itself (that is, the correlation between two or more behaviours across multiple individuals) is a property of a population, not of an individual (Bell, 2007). Although behavioural syndromes can be affected by external factors in the environment (Martins & Bhat, 2014), there must also be variation between individuals for behaviours to be correlated at the population level. Selection can favour different behavioural strategies depending on the context. Variation in conditions and context may help explain the conservation of multiple different strategies (Sih et al., 2004a).

1.1.1 *Movement syndromes*

In many animal species, individuals show consistent (individually stable and repeatable) variation in activity and movement. For example, great tits (*Parus major*) show repeatable levels of exploratory behaviour (Dingemanse et al., 2002) and northern pike (*Esox lucius*) show consistency in migratory timing between years (Tibblin et al., 2016). These patterns suggest the potential presence of behavioural syndromes related to movement (hereafter “movement syndromes”). Because of logistical challenges associated with studying large-scale movement in free-living animals, many studies have involved monitoring movement over small geographic scales or in captivity. For example, Dingemanse and de Goede (2004) studied exploratory behaviour and dominance in great tits in a laboratory setting and found fast exploring birds occupied the lowest dominance ranks. However, movement syndromes can also include long-range movements, as migration distance and natal dispersal has shown to be positively correlated in song sparrows (*Melospiza melodia*; Kelly et al., 2016).

1.2 *Migratory behaviour*

Seasonal migration is an important life history trait for many animals, often caused by environmental changes associated with seasonal variation in conditions and resources (Pulido, 2007). During this large-scale movement, individuals may encounter different environments with varied levels of food quality, competition and predation (Carbó-Ramírez & Zuria, 2015). Many migratory birds show differential migration, meaning that individuals, sexes or age classes vary in migratory distance (Jenkins & Cristol, 2002; Pulido, 2007). For example, male white-throated sparrows (*Zonotrichia*

albicollis) often overwinter closer to the breeding grounds (corresponding to shorter migration distances) than do females (Jenkins & Cristol, 2002). This pattern presumably reflects sex differences in the costs as well as the benefits of overwintering close to the breeding grounds. Males are often larger than females and may be better able to withstand harsh conditions; male fitness may be more closely associated with the ability to arrive earlier at the breeding grounds (Tonra et al., 2011; Kelly et al., 2016; Lymburner et al., 2016). For example, female ospreys (*Pandion haliaetus*) were found to migrate approximately 2000 km and 1500 km farther on average than males during migration in Eastern and Midwestern North American populations, respectively (Martell et al., 2001).

Like many behaviours, variation in migration distance is likely the result of trade-offs between the costs and benefits of long versus short migrations. Migrating further distances can be energetically costly but allow reaching better quality wintering habitats, while migrating shorter distances may require the individual to overwinter in harsh conditions, potentially compromising breeding condition, but allow earlier return to the breeding grounds (Tonra et al., 2011). The costs and benefits of both longer and shorter migration distances may help to explain the persistence of extensive variation in this trait, even among individuals of the same age and sex.

The closest ancestors of migratory species were sedentary, and migration has been proposed to have evolved through colonization of new areas following the most recent post-glacial maximum (Pulido, 2007). Thus, long-distance migratory movements may have evolved as a by-product of other movement behaviours such as dispersal or exploration, suggesting that movement syndromes involving migration distance are likely to exist. Indeed, migration distance in song sparrows (*Melospiza melodia*), inferred from

stable isotope analysis, is positively associated with juvenile dispersal tendency, inferred from microsatellite genotypes and genetic assignment tests (Kelly et al., 2016).

1.3 Exploratory behaviour

Generally occurring on a much smaller geographic scale than migration, exploration is a spontaneous behaviour used to gain knowledge of the environment (Renner, 1990; Huang et al., 2016). Exploration allows animals to find foraging patches, territories and hiding spots (Verbeek et al., 1994), and gauge risks in the environment (Huang et al., 2016). In young birds, it may be used to create a mental map of the natal site (Mukhin et al., 2005), and therefore may aid birds in finding the breeding grounds upon return from migration the following year. As for migration distance, the level of exploratory behaviour differs substantially between individuals even within the same species. Age and sex differences in exploration may vary from species to species.

Studies in exploration of great tits have not shown age differences between juveniles and adults (Drent et al., 1996; Dingemanse et al., 2002), whereas juvenile chimango carcaras (*Milvago chimango*) were found to display more exploratory traits than the adult raptors (Biondi et al., 2010). In terms of sex, male and female common toads (*Bufo bufo*) differ in their exploration methods (Ogurtsov et al., 2018), while zebra finches (*Taeniopygia guttata*) only show differences in exploratory consistency between the sexes (Schuett & Dall, 2009). The importance of exploratory behaviour suggests that this variation may influence survival (Verbeek et al., 1994).

Higher levels of exploration may be adaptive in individuals that are socially subordinate. If they are displaced from preferred areas by dominant individuals, subordinate individuals that are more prone to exploration may be better able to find resources elsewhere (Verbeek et al., 1994). Behavioural syndromes linking exploration to social dominance or aggression may help to explain the persistence of variation in exploratory behaviour within a species. When resources are rich, more aggressive and territorial individuals may benefit from these traits when defending high quality territories, whereas when resources are scarce, more subordinate and exploratory birds will likely have the advantage in finding foraging patches (Dingemanse & De Goede, 2004). Individuals within a population can also vary in the nature of their exploratory behaviour. In a study of the exploratory habits of great tits, Verbeek et al. (1994) noted two types of the behaviour: fast and superficial explorers and slow and thorough explorers. They described fast and superficial explorers as individuals who quickly visited features in a novel environment but may have paid little attention to the environment itself, whereas the slow and thorough explorers remained more alert and cautious when navigating the environment (Verbeek et al., 1994). In this situation, fast and superficial explorers may be better able to exploit resources when competition is high but resources are abundant, while slow and thorough explorers may be better able to find resources when competition is low but resources are scarce (Sih et al., 2004a).

Much exploratory behaviour, although not all, is done in the context of foraging. Individuals that explore the environment to forage in places beyond what are normally the most profitable areas may be better suited to changing environments, whereas individuals that forage consistently in the same profitable locations may be better suited

to stable environments (Verbeek, et al., 1994). This variation in exploration phenotypes may translate to performance in other contexts, such as during migration. Because migratory birds encounter different habitat types during stopover, and because longer migrations may also be associated with a greater difference between breeding and wintering habitats, selection favouring increased migration distance might also favour increased exploration.

Exploratory behaviour is often tested using a novel environment, which is an effective and repeatable method of assessing exploration (Dingemanse et al., 2003; Huang et al., 2016). The fact that exploratory behaviour is repeatable, i.e., varies consistently among individuals over repeated measurements, suggests that it represents a stable and consistent behavioural trait. Exploratory behaviour in great tits is associated with traits such as boldness and aggression (Drent et al., 1996; Dingemanse et al., 2003), as well as movements including natal dispersal (Dingemanse et al., 2003). Fast and more superficial exploring birds in other studies have been found to be more aggressive but may be socially dominant or a subordinate, while slow and more thorough explorers display lower levels of aggression while being intermediates in terms of dominance (Marchetti & Drent, 2000).

Associations between exploratory behaviour and natal dispersal in great tits (Dingemanse et al., 2003), combined with previous findings in song sparrows that seasonal migration and natal dispersal are correlated (Kelly et al., 2016) suggest that these movements may be part of a single behavioural syndrome. Some aspects of these movement behaviours may be linked, meaning that they are not fully independent traits. Exploratory flights may also be used by birds to orient with landmarks near to them (such

as major bodies of water) prior to migratory departure, and to detect meteorological conditions and assess the risk of continuing migration in potentially dangerous conditions (Schmaljohann et al., 2011). A relationship between feeding, exploration, and orientation has been suggested in sedge warblers (*Acrocephalus schoenobaenus*), where leaner birds ate and explored more, and also oriented better in Emlen funnel tests than fatter individuals in preparation for autumn migration (Marchetti & Zehindjiev, 2009). As noted above, if migration is the derived state and evolved from ancestral sedentary species (Pulido, 2007), exploration may have been a precursor to the development of migratory behaviour. If these two behaviours are related, there may be common mechanisms influencing both behaviours.

1.4 Proximate causes of variation in movement behaviour

1.4.1 Androgens

Hormones play a role in many aspects of animal behaviours, such as aggression, territoriality, migration, and novelty seeking (Moore, 1984; Wingfield & Soma, 2002; Garamszegi et al., 2008; Tonra et al., 2011; Lymburner et al., 2016). Hormone levels can affect behaviours in all life history stages including early-life development, breeding, migration and non-breeding (Wingfield & Soma, 2002). In particular, much attention has focused on androgens—important steroid hormones that regulate behaviour and reproduction in many animals. Androgens such as testosterone circulate in both males and females, and play an important role in aggressive behaviours (Moore, 1984). Testosterone levels can vary greatly among individuals during the breeding season, and high levels of plasma testosterone are correlated with increased aggression in territorial

disputes of song sparrows (Wingfield & Soma, 2002). High levels of circulating androgens can also be costly to animals. Consistently high androgen levels can cause immunosuppression, reduction in fat stores, increase rate of injury, and decrease parental care in males (Hegner & Wingfield, 1987; Dufty, 1989; Ketterson & Nolan, 1992; Folstad & Skarstein, 1997).

Identical behaviours can occur in multiple life history stages, and a behaviour mediated by a particular hormone in one life history stage is likely to be mediated by the same hormone in another stage (Wingfield & Soma, 2002). Similarly, if selection on one androgen dependent trait (e.g. territoriality) alters the population average circulating androgen concentrations, other androgen-dependent traits (e.g. immune function) will likely be co-selected and also change as a result (Garamszegi et al., 2008). Androgens have important implications for both behavioural strategies and differences in individual physiology, meaning that selection on an animal's behaviours may have pleiotropic effects on their physiological traits that drive their behaviours (van Oers et al., 2011).

Variation among individuals in circulating androgen levels may contribute to variation among individuals in the propensity for movement behaviours such as exploration and migration. If androgen levels represent a shared mechanism contributing to movements at both small (exploration) and large geographic scales (migration) this could result in correlations between these two movement behaviours. In both male and female song sparrows, androgen concentrations decrease with increasing migration distance (even though males have higher circulating androgen concentrations than females), and individuals with high androgen levels on the breeding grounds also migrated shorter distances (Lymburner et al., 2016). In addition, earlier arriving male

birds have shown higher total and plasma androgen levels than later arriving males (Tonra et al., 2011).

Certain concentrations of androgens may be required for migration because without them, individuals would not be able to facilitate preparation for migratory readiness, such as regulating fattening (Garamszegi et al., 2008). Higher circulating levels of androgens may also allow individuals to return to the breeding grounds earlier and be in better breeding condition upon return (Tonra et al., 2011), possibly offsetting some costs of overwintering in a harsher environment due to migrating shorter distances. Circulating androgen levels of spring-captured song sparrows are negatively associated with past migration distance (i.e. previous winter's latitude; Lymburner et al., 2016), suggesting a potential role for androgens in mediating migration distance. Androgens may also influence exploratory behaviour, given that they influence other behaviours such as aggression and boldness (Wingfield & Soma, 2002; Dingemanse et al., 2003), and have been suggested to affect novelty seeking behaviours in other studies (van Oers et al., 2011). Testosterone (both at baseline and peak levels) has been found to be repeatable within species (Garamszegi et al., 2005), suggesting that androgen levels may be a consistent trait that is repeatable over time. If androgens are influencing movement on one geographic scale, it may show a similar relationship to other movement patterns on another, smaller scale.

1.4.2 Dopamine receptors

In addition to androgens, other mechanisms may mediate the relationship between movement behaviours. Migratory restlessness has been shown to be a heritable trait in

European blackcaps (*Sylvia atricapilla*), as this behaviour was correlated between parents and offspring (Berthold et al., 1994), though the mechanism for this remains largely unexplored. Additionally, the urge to migrate, as well as orientation behaviours, can be transmitted into a nonmigratory population, suggesting a substantial genetic basis (Berthold et al., 1990), and this can spread under the right environmental conditions. In some species, it only takes three generations of selective breeding to turn a partially migrating population to either completely migratory or exclusively sedentary (Berthold, 1988). In addition, genes such as *Adcyap1* encode for polypeptides that play a role in circadian and circannual timing that have been found to regulate migratory behaviour in some species (Bazzi et al., 2016).

Novelty seeking behaviours such as exploration likely have a genetic component as well. Multiple studies have shown evidence that exploratory behaviour is a heritable trait (Dingemanse et al., 2002; Drent et al., 2003). Variation in the *DRD4* dopamine receptor gene has been proposed to affect novelty seeking behaviours in organisms across taxa, including mammals such as mice (Helms et al., 2008) and humans (Cloninger et al., 1996), as well as in a number of avian species (Fidler et al., 2007; Mueller et al., 2014; Timm et al., 2019). Because of its involvement in the dopaminergic reward system, the *DRD4* gene may be linked to novelty seeking and activity behaviours such as exploration (Mueller et al., 2014; Verhulst et al., 2016; Timm et al., 2019).

The *DRD4* gene encodes for a protein receptor domain that accepts the neurotransmitter dopamine, which is related to motivational behaviour (Netter, 2006). Polymorphisms within this gene have been found associated with variation in exploratory behaviour (Fidler et al., 2007; Garamszegi et al., 2014; Timm et al., 2019). Though the

single nucleotide polymorphisms (SNPs) found to be associated with exploration in these populations are often synonymous (Garamszegi et al., 2014; Mueller et al., 2014; Timm et al., 2019) and thus do not have direct effects on protein structure, they can still have effects on other processes such as mRNA translation and stability that can alter protein structure and affect expression (Duan et al., 2003; Chamary et al., 2006). Epigenetic factors such as methylation at the DRD4 gene can also have an effect on the degree to which animals explore (Verhulst et al., 2016), suggesting this gene's link to the reward pathway has strong influence on novelty seeking behaviours.

One way in which behavioural syndromes can evolve is if the variation in two or more behaviours are influenced by the same proximate mechanisms, such as genetic variation and/or variation in circulating hormone levels (Garamszegi et al., 2014). One genetic component controlling hormones can have pleiotropic effects on other independent behaviours if they are evolutionarily linked through the same mechanism (Garamszegi et al., 2014). The potential relationship between migratory and exploratory behaviours may be the rooted in genetic and hormonal connections.

1.5 Study system

The migratory patterns of the Eastern Song Sparrow (*Melospiza melodia melodia*) are well described. This subspecies overwinters all across southeastern Canada and the eastern United States, spanning from southern Ontario and northern states such as Michigan and New York, to southern states such as Louisiana and Florida (Davis & Arcese, 1999). These birds show significant variation in migratory distances (Davis & Arcese, 1999), even among individuals breeding at the same location during spring and

summer (Kelly et al., 2019), making them an excellent model species for studying migratory behaviour.

Though the exploratory behaviour of song sparrows has not been well studied, research has been conducted that suggest song sparrows display behavioural syndromes. Aggression and boldness vary between both individuals and populations of song sparrows, and a positive relationship has been demonstrated between the two behaviours (Evans et al., 2010; Myers & Hyman, 2016). In other species, these traits have been found to be correlated with exploration (Drent et al., 1996).

Song sparrows in my study population breeding near Newboro, Ontario have been studied for well over a decade, and they are known to display differential migration (Kelly et al., 2016, 2019; Lymburner et al., 2016). This variation in latitudinal migration distance raises the possibility that exploration tendency is similarly variable, and that these two movement behaviours might be correlated, such that different individuals have consistently different movement strategies across multiple scales. Moreover, circulating androgen concentrations in this study population of song sparrows have been demonstrated to show a negative relationship with migration distance; individuals who migrated shorter distances had higher androgen concentrations in their plasma (Lymburner et al., 2016).

1.6 Objectives and hypothesis

In this thesis, my objectives were to test for (1) a relationship between the level of exploratory behaviour and migration distance in song sparrows, and (2) whether one or more proximate mechanisms contributes to variation in both these behaviours. I captured

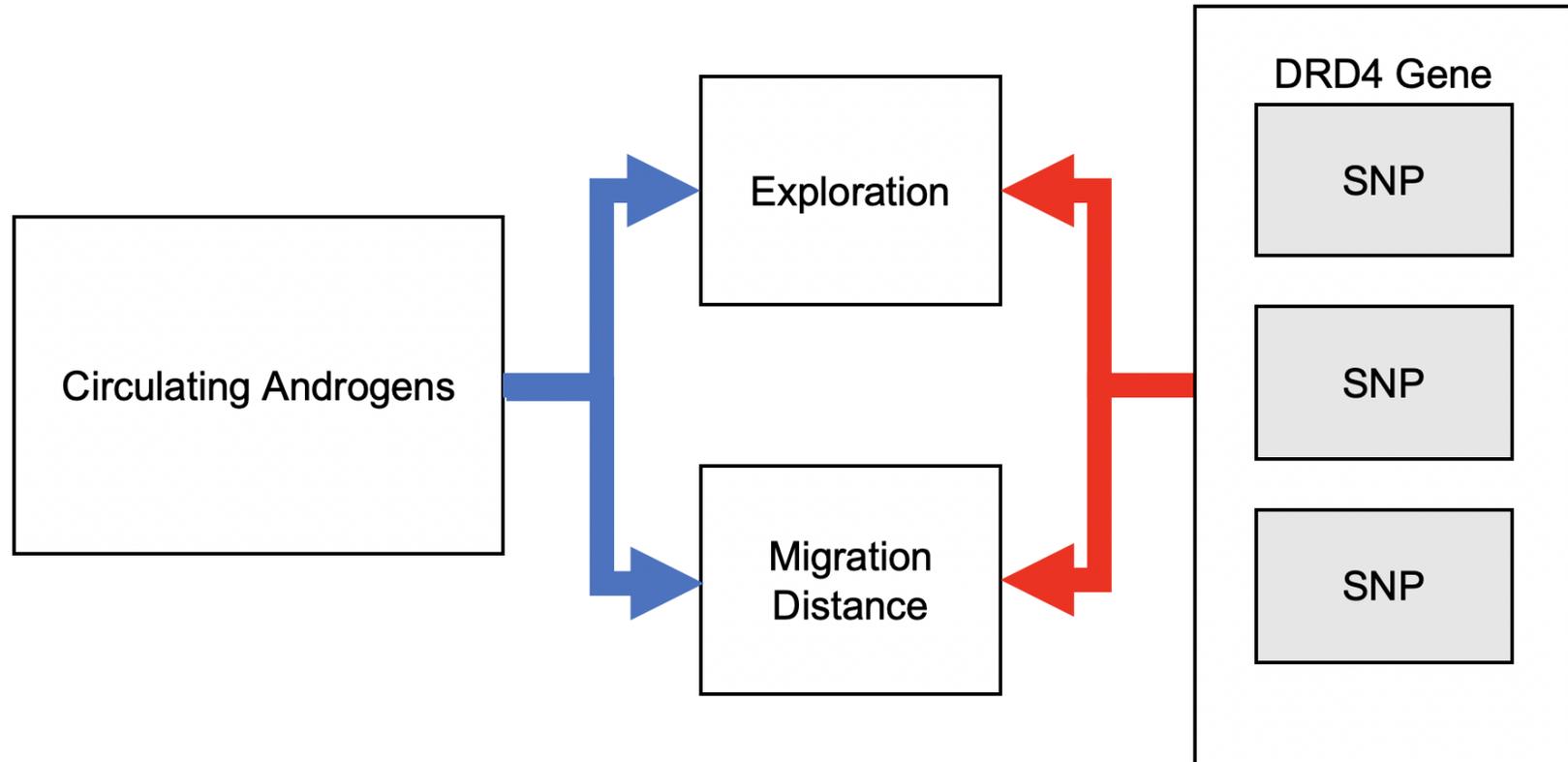
individual adult song sparrows shortly after their return to the breeding grounds in spring, tested them in a novel environment room to measure exploratory behaviour, and used stable isotope analysis of winter-grown claw tissue to infer the relative overwintering latitude, and therefore the migration distance, of the same individuals. To examine potential common proximate mechanisms, I tested whether circulating androgen concentrations predicted both exploratory behaviour and migration distance; I also tested whether both these movement behaviours were associated with DNA sequence variation at the dopamine receptor locus DRD4.

I hypothesized that there is a common hormonal and/or genetic mechanism that underlies both small- and large-scale movement patterns. If these small- and large-scale movement behaviours share a common mechanism they should be positively correlated, thus I also hypothesized that migratory birds show a behavioural, or ‘movement’ syndrome including both exploration and migration. To test this hypothesis, I examined the correlation between exploration and migration distance. Several candidate mechanisms might underlie, and thus generate correlations between, these two movement behaviours: in this thesis I focused on circulating androgen levels and variation in the dopamine receptor DRD4 gene. I tested the relationship between circulating androgen levels and each of the two movement behaviours, as well as the relationship between DRD4 sequence variation and each of the two movement behaviours. If either potential mechanism is associated with both movement behaviours, this would implicate it as a common mechanism that may explain the relationship between small- and large-scale movements. (Figure 1.1).

To my knowledge, the relationship between the small- and large-scale movement behaviours of exploration and migration has not been studied previously, meaning the results of this study provide novel information about the association of movement of an avian species on different geographic scales. Moreover, the proposed candidate mechanisms linking these two behaviours have only been tested previously for their effect on single behaviours. The effect of circulating androgens on exploratory behaviour and the association between variation in DRD4 and migration have not been extensively studied before. Examining the relationship between exploration and migration and identifying proximate mechanisms that might underlie any relationship between them, gives a better understanding of the evolution of both these key life history traits.

Figure 1.1. Proximate mechanisms hypothesized to mediate the potential relationship between exploratory behaviour and migration distance.

I hypothesized that there is a common set of hormonal or genetic mechanisms that affect small- and large-scale movement patterns in song sparrows (*Melospiza melodia*). I examined two potential candidate mechanisms, circulating androgen concentrations and sequence variation at a dopamine receptor locus, that may affect movement. If a correlation is found between exploratory behaviour and migration distance, these movement behaviours may have a shared hormonal and/or genetic basis.



2. *Methods*

2.1 *Study animals and field methods*

All animal procedures were approved by the University of Western Ontario's Animal Care Committee (protocol 2016-017). My study focused on a population of song sparrows (*Melospiza melodia*) that breed near Newboro, Ontario (44.66° N/76.22° W) on land owned by the Queen's University Biological station. This population has been studied for over 15 years and thus age and breeding history is known for most individuals (Kelly et al., 2019). Studies of latitudinal migration distance in this population (as inferred from stable isotope analysis of winter-grown claw tissue; Wassenaar & Hobson, 2000) have shown substantial variation among individuals. Values of claw stable isotopes correspond roughly to wintering latitudes between Florida and Pennsylvania, U. S. A. (Kelly et al., 2016, 2019). Among individuals sampled in multiple years, moreover, latitudinal migration distance is repeatable (Kelly et al., 2016). Individuals in the sampled population also show breeding-site fidelity, generally returning to the same breeding territory or one within a hundred metres of that used the previous year

In April (15th) to May (15th), 2018 and 2019, along with other members of the research team, I captured adult (after hatch-year) song sparrows in seed-baited Potter traps between 0630 and 1130. If not already banded, we outfitted each individual with a numbered aluminum Canadian Wildlife Services leg band and a unique combination of three coloured plastic leg bands in order to discern between individuals. We determined sex in the field by the presence (male) or absence (female) of a cloacal protuberance or the presence of a brood patch (incubating female) supplemented by measurements of unflattened wing length and tarsus (measured with dial calipers; ± 0.1 mm). Sex was later

confirmed through genetic analysis using primers P2 and P8 (Griffiths et al., 1998). We measured the mass of each bird (to the nearest 0.2 grams) using a spring scale. Age was inferred from banding records of previous years: birds first captured and banded as adults are assumed to be one year of age upon first capture (hereafter second-year), whereas birds that were previously banded as adults in past breeding seasons are known to be two or more years old (hereafter after-second-year).

We collected small claw samples (~2.5 mm) from each hallux (back) claw, for stable isotope analysis of latitudinal migration distance (see *Stable Isotope Analysis* below). Based on previous work measuring claw growth rates of the closely related white-throated sparrow, (*Zonotrichia albicollis*; Kelly et al., 2016) this section of claw is expected to correspond to tissue grown between October and January, i.e., on the wintering grounds. Blood samples (~200 μ L) were collected through brachial venipuncture within 10 minutes of capture. A small amount (~25 μ L) of blood was blotted onto filter paper saturated with 0.5 M Na-EDTA (pH 8.0) preservative for genetic confirmation of sex, and DRD4 genotyping (see *Genetic analysis*, below). The remainder of the blood samples were kept cool on ice for several hours while in the field, then spun down in a microhematocrit centrifuge to isolate plasma for androgen assays (see *Androgen assays*, below).

2.1.1 *Novel environment test*

Immediately after field processing and handling as described above, each animal was placed in its own covered holding cage (34 x 28 x 48 cm) and held for at least 1 hour to habituate. Each cage contained two wooden perches, food (millet) and water, and was

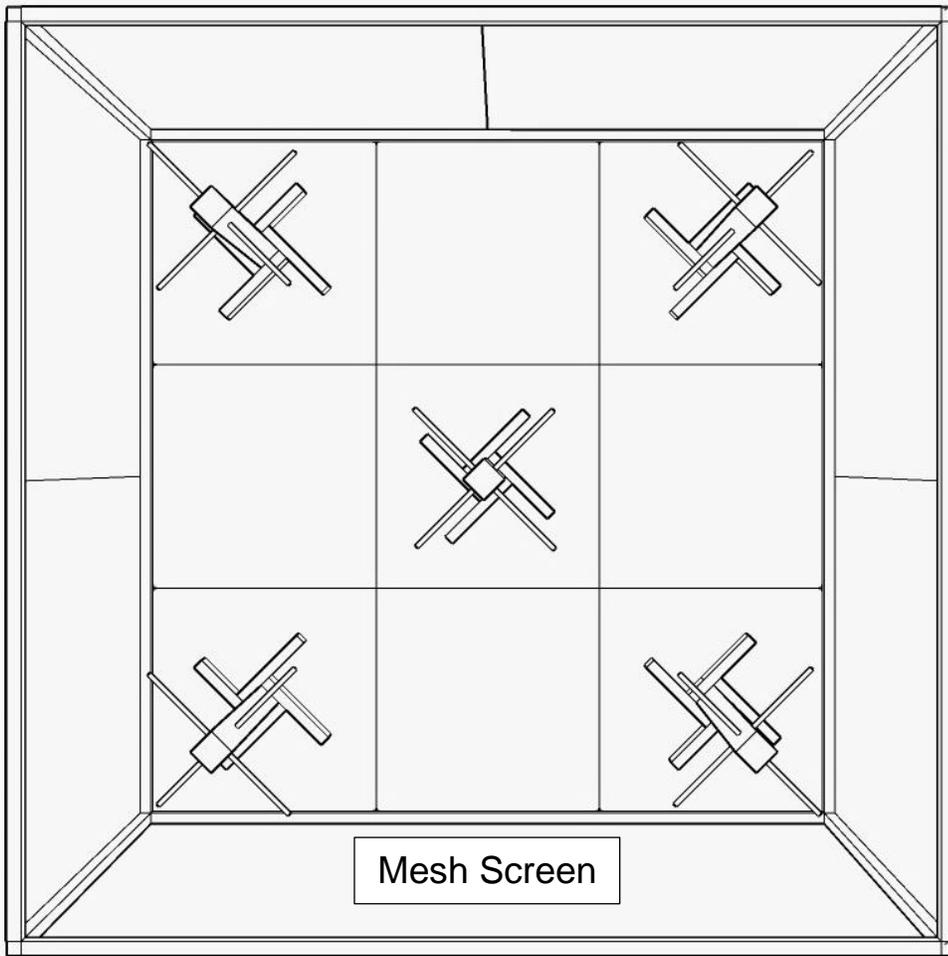
covered by cotton sheeting in order to calm and quiet the bird. After the habituation period, the bird was then released (without handling) into the ‘novel environment room’. This outdoor structure had a footprint of 2.4 x 2.4 m and was 1.8 m in height, placed on a flat concrete pad. Three of the four walls, as well as the ceiling, were lined with translucent white sheets of corrugated plastic to prevent the animal seeing outside of the structure, while providing near-natural lighting conditions inside. The fourth wall was lined with a transparent mesh screen with a canopy covering the area opposite the screen. Three sides of the canopy were lined with tarps so that the external environment was not visible from inside the novel environment room.

The testing room contained five artificial ‘trees’ (wooden posts, 1.2 m in height) each with 4 ‘branches’ (wooden perches, ~20 cm long, arranged at different heights and angles along the ‘tree’) and a base providing four additional perches (wooden planks, 5.1 cm x 10.2 cm x 30.5 cm; Figure 2.1). Trees were arranged in the room with one tree in each of the four corners of the room and the fifth tree in the centre. The orientation of the trees did not change between trials. Under the canopy, outside of the novel environment room, a video camera (Activeon CX Action Camera) was positioned on a tripod to record the exploration trial. The camera was positioned in such a way that the entirety of the experimental area was visible. The floor of the room was lined with a white tarp that was marked to display a 3x3 grid, resulting in the identification of nine sectors (0.8 m by 0.8 m). The dimensions, location, orientation, tree arrangements and other features of the novel environment room were identical for both years of the study.

After habituation, I introduced the focal bird to the testing structure without handling and without allowing the birds to see the experimenters by sliding open the

Figure 2.1 Top down view of the novel environment room used to test exploratory behaviour

Novel environment room used to test exploratory behaviour in song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. The room had a footprint of 2.4 x 2.4 m and a height of 1.8 m. Sectors are 0.8 m x 0.8 m for scale.



doors of holding cage and testing structure. Birds were tested individually so that their behaviour was not influenced by the presence of other birds in the novel environment room. Behaviours were recorded with a video camera for ten minutes, with experimenters not visible to the birds during trials. After ten minutes of exploration, the bird was released. The location of the novel environment room was within 40-750 m of all capture locations, allowing for easy return to their territories upon release. For animals that were recaptured at least seven days after their first behavioural trial ($n = 37$), I conducted a second trial using the procedures described above, to assess repeatability of exploratory behaviour. In all, I recorded a total of 130 trials, using a total of 80 unique birds. 50 birds had a second trial conducted, either within the same calendar year ($n = 37$) or in 2019 as well as 2018 ($n = 13$).

After the field season, I scored each bird's video and recorded the number of flights, number of hops, head scanning rate (the number of head scans divided by the total time the bird was stationary), number of trees visited, number of features visited (i.e., trees, perches, walls, floor and ceiling), number of sectors visited, and latency to visit the first new feature. These behaviours were chosen based on previous studies of exploration in other avian species (Dingemanse et al., 2003; Huang et al., 2016). Videos were scored blind with respect to latitudinal migration distance and bird identity.

2.2 *Stable isotope analysis*

I estimated latitudinal migration distance using stable isotope analysis of the nonexchangeable hydrogen of winter-grown claw tissue. This analysis was conducted at Western University's Laboratory for Stable Isotope Science – Advanced Facility for

Avian Research. Because of a latitudinal gradient across eastern North America in amount-weighted growing-season precipitation, such that $\delta^2\text{H}$ (deuterium) increases with decreasing latitude (Wassenaar & Hobson, 2000), claw tissue can be used to estimate the geographic location in which it was formed during a previous season (Mazerolle & Hobson, 2005). Although $\delta^2\text{H}$ can be measured in both feathers and claws (Mazerolle & Hobson, 2005), song sparrows molt their primary feathers on the breeding grounds, not the wintering grounds (i.e. pre-basic molt occurs before fall migration, and these birds have no pre-alternate molt). This molt timing makes the feathers of song sparrows uninformative as to wintering origins (Arcese et al., 2002).

Sections of the hallux claws of each individual were cut with sharp scissors in the field, then stored in plastic microcentrifuge tubes for several months awaiting stable isotope analysis. Claw sections were then placed in glass vials and 600 μL of a 2:1 chloroform methanol solution was added to each vial. Vials were then sealed and shaken in order to clean the claws and remove any dirt or other material that may be adhered to them. Once every vial was filled and inverted, the chloroform methanol solution was pipetted out and the vials were placed uncapped in a fume hood overnight to allow the remaining liquid to evaporate, leaving only the sterilized claws. The vials were then capped until they were weighed. Each claw was weighed to 0.350 ± 0.020 mg (Mettler Toledo MX5 Microbalance PSU30A-3, Griefensee, Switzerland) and enclosed in a silver capsule. Capsules were placed in a sterile 96-well plate and stored at room temperature in a desiccator. If a claw section had a mass below the acceptable range of 0.350 ± 0.020 mg [N=1 (2018), N=0 (2019)], it was not analyzed.

$\delta^2\text{H}$ analysis was conducted using continuous flow isotope ratio mass spectrometry performed on a high temperature conversion elemental analyzer with isotope ratio mass spectrometer (Thermo scientific, Bremen, Germany). Analyses of claws collected in 2018 were conducted using a chromium reactor (1120 °C), and 2019 claw analysis was conducted using a glassy carbon reactor (1450 °C). Isotope values were calibrated using two reference standards (Kudu Horn Standard [KHS]: -35.3 ‰, and Caribou Hoof standard [CBS]: -157.0 ‰; Coplen, 2017a,b). All keratin $\delta^2\text{H}$ results are reported in units per mil (‰) with an analytical precision of ± 2 ‰ and normalized on the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale. $\delta^2\text{H}$ values are interpreted as relative latitudinal migration distance, such that less negative values are associated with more southerly overwintering latitude (greater migratory distance). One individual captured in 2018 did not have a claw sample with enough mass for stable isotope analysis and was therefore excluded from all further analyses. In all, I performed stable isotope analysis on claw samples from a total of 79 unique individuals (32 in 2018 and 47 in 2019). For individuals caught in both 2018 and 2019, the first stable isotope values of their claws (i.e., only values from 2018) were used in further analyses, corresponding to the year of their first exploratory trial.

2.2.1 Estimating total migration distance with isoscape assignment

Stable-hydrogen values within tissues can be compared to predictable geospatial patterns of naturally occurring stable isotopes, called isoscapes, in order to infer origin of migratory animals (Hobson & Wassenaar, 2019). I used a raster layer of amount-weighted growing-season precipitation $\delta^2\text{H}$ values (months when mean daily temperature

was $>0^{\circ}\text{C}$; Bowen et al., 2005) using the R package ‘raster’ (version 3.0-7; Hijmans, 2019b). The isoscape was converted into a predicted tissue isoscape using the relationship between isotope ratios of environmental waters and animal tissues produced at known sites (Hobson & Wassenaar, 2019). For the song sparrow, I used the calibration equation for the ‘short-distance migrant ground forager’ guild ($\delta^2\text{H}_f = -36.9 + 0.95 \delta^2\text{H}_p$; Hobson et al., 2014) with an algorithm ($\delta^2\text{H}_f = 24.1 + 1.3 [\delta^2\text{H}_c]$) to convert the song sparrow $\delta^2\text{H}_c$ values to $\delta^2\text{H}_f$ equivalents due to the lack of an existing $\delta^2\text{H}_c$ isoscape (Kelly et al., 2019).

Song sparrow range data from BirdLife International (2019) was used to restrict the isoscape to the potential region of origin for overwintering birds using the ‘mask()’ function from the R package ‘raster’ (version 3.0-7; Hijmans, 2019b). I used the permanent and overwintering ranges of the song sparrow and restricted the extent to a specific range of latitudes ($25^{\circ}\text{N} - 46^{\circ}\text{N}$) and longitudes ($65^{\circ}\text{W} - 95^{\circ}\text{W}$) because this range was estimated to encompass the entirety of the known migratory range of the population (based on previous measurements and recapture data; Kelly et al., 2019; S.A MacDougall-Shackleton unpublished data). For each individual $\delta^2\text{H}_c$, I assessed the likelihood that a given cell within the predicted feather isoscape represented the potential origin using a normal probability density function adapted from Van Wilgenburg and Hobson (2011):

$$f(y^*|\mu_c\sigma_c) = \left(\frac{1}{\sqrt{2\pi}\sigma_c}\right) \exp\left[-\frac{1}{2\sigma_c^2}(y^* - \mu_c)^2\right]$$

where $f(y^*|\mu_c\sigma_c)$ represents the probability that a given cell (c) within the $\delta^2\text{H}$ isoscape represents a possible origin for a song sparrow of unknown origin (y^*), given the

expected mean $\delta^2\text{H}$ for that cell (μ_c) from the calibrated $\delta^2\text{H}$ isoscape, and the expected standard deviation (σ_c) of $\delta^2\text{H}$ between individuals growing their claws at the same locality. I used a standard deviation of residuals of 18.4 ‰ (Hobson et al., 2012) to estimate σ_c . Lastly, I used an odds ratio of 2:1 to select the upper 67% of cells, creating a binary surface representing the likely origins of each individual (Van Wilgenburg & Hobson, 2011).

The centroid of each bird's likely overwintering range was calculated and the distance from the breeding grounds to each centroid was determined as a measure of the estimated total migration distance (in km) of each bird using the R package *geosphere* (version 1.5-10; Hijmans, 2019a). This measurement is referred to hereafter as "total migration distance" because it provides a distance estimate taking into account longitudinal variation in addition to the latitudinal variation inferred by the stable isotope values of each individual's claw sample.

2.3 *Androgen assay*

Blood samples collected in the field were kept on ice for at most 4 hours before they were spun down in a microhematocrit centrifuge at 10,000 rpm for 10 minutes to separate plasma from hematocrit. Plasma was then collected using a Hamilton syringe, and dispensed into sterile 0.5 mL screw top microcentrifuge tubes outfitted with O-rings to prevent evaporation and stored at $-20\text{ }^\circ\text{C}$.

Levels of plasma androgens were assayed using an enzyme immunoassay kit (1–2403, Salimetrics, State College, PA, U. S. A.) that has been previously validated for use in song sparrows (Schmidt et al., 2014). The kit cross-reacts with dihydrotestosterone as

well as testosterone, thus the hormone levels measured are referred to as androgens as opposed to solely testosterone. In all, I quantified circulating androgen concentrations for a total of 78 song sparrows ($N_{\text{Males}} = 39$, $N_{\text{Females}} = 39$).

The assay is conducted according to the manufacturer's instructions, except that all of the plasma samples are first diluted 1:5 with the assay buffer diluent and the samples are analyzed in duplicate by adding 25 μL of diluted plasma to each well. Androgen concentration values that initially measured above 1000 pg/mL were further diluted threefold (to a final dilution of 1:15) and re-analyzed, to ensure that measurements fell within the recommended standard range (i.e. linear portion of the standard curve). After the androgen levels were measured and concentration estimates were produced, the concentrations were adjusted according to their dilution factor (5 or 15). The mean intra-assay coefficient of variance among all plates was 6.91%, and the inter-assay coefficient of variance was 18.4%. Multiple high and low controls provided in the kit were used for the plates, as many randomized samples were run and the volume of controls needed was higher than provided with one plate, potentially elevating the inter-assay coefficient of variance.

2.4 Genetic analysis of *DRD4* exon 3

2.4.1 *DRD4* exon 3 amplification

DNA was extracted from blood blots using an ammonium acetate protocol to salt out proteins. One individual's DNA was unable to be extracted and was thus excluded from further genetic analyses. DNA quantity was measured using a UV-vis spectrophotometer (Thermo Fisher Scientific Nanodrop 2000) and concentrations were

adjusted to 20 ng/ μ L. Exon 3 of the DRD4 gene is known to have single nucleotide polymorphisms (SNPs) that may be related to variation in exploratory behaviour in other avian species (Fidler et al., 2007; Mueller et al., 2014). Accordingly, I used previously published primers (DRD4_I2F, DRD4_I3R; Garamszegi et al., 2014) for polymerase chain reaction (PCR) amplification of this region. The primers were used to amplify the whole of exon 3, with an expected product size of ~600 bp.

Polymerase chain reaction (PCR) reagents were added to a labelled 1.5 mL microcentrifuge tube to serve as the master mixture to be added to each sample. The total volume of the reaction mixture for each sample was 10 μ L, and included 1x PCR buffer (Invitrogen, Burlington, Canada; 200 mM Tris-HCl pH 8.4, 500 mM KCl), 2 mM MgCl₂, 0.2 mM dNTPs (Invitrogen), 0.2 mM DRD4_I2F and DRD4_I3R primers (Invitrogen), 0.5 U *Taq* polymerase (Invitrogen), and approximately 25 ng of genomic template DNA. Once all reagents were added, each tube was placed in a thermocycler (Eppendorf Mastercycler, Hamburg, Germany) to amplify the target gene region using the following reaction conditions: 1) 94 °C for 1 minute, 2) 94 °C for 30 seconds, 3) 56 °C for 45 seconds, 4) 72 °C for 45 seconds, 5) return to step 2 and repeat 27 times, 6) 72 °C for 5 minutes, 7) hold temperature at 4 °C.

Confirmation of DNA amplification was conducted through gel electrophoresis. A 2% agarose gel was made using agarose (2g) and 1X TBE buffer (100mL). RedSafe dye (5 μ L) was added and mixed into the gel before allowing it to set. PCR product (5 μ L) from each sample was mixed well with tracking dye (1 μ L) and deposited into the corresponding well in the gel. When all samples had been added, 1kb DNA ladder (5 μ L) was deposited into a well in the gel to compare amplified DNA fragment lengths and

verify that the correct fragment of DNA had been amplified according to the expected DNA fragment length. The gel was run at 96 V for ~1.5 hours. Gels were imaged using ultraviolet light on a Bio-Rad Gel Doc 2000 and samples with confirmed amplification were cleaned for sequencing (see below). Individual's DNA that did not extract and amplify [N=0 (2018), N=1 (2019)], were not used in further genetic analyses.

2.4.2 Sequencing

Once amplified, PCR products were cleaned for sequencing. To clean the samples, a reaction mixture was created with each sample, consisting of PCR product (5 μ L), Exonuclease 1 (0.5 μ L, Thermo Scientific) and Fast AP (1 μ L, Thermo Scientific). The mixture was centrifuged briefly to 10,000 RPM and then incubated for 15 minutes at 37 °C, after which the reaction was stopped by heating the mixture to 85 °C for 15 minutes. The concentration of each cleaned sample was then measured using a NanoDrop, and the samples were diluted to 20 ng/ μ L in a new labelled 1.5 mL microcentrifuge tube using 1x TE Buffer. Once diluted, DRD4_I2F primer (2 μ M, 5 μ L) was added to each tube. The prepped DNA samples were then sent to the Robart's Research DNA Sequencing Facility (London, Ontario) to be sequenced.

2.4.3 Surveying for SNPs

The resulting sequences were aligned using BLAST to verify that the amplified segments of DNA were indeed the target region. The sequenced portions were confirmed to be the exon 3 of the DRD4 gene as they had >94% identity matches to orthologs from many other avian species including *Parus major*, *Prunella modularis*, and *Zonotrichia*

albicollis, among others. Sequences from all individuals were aligned to the great tit (*Parus major*) ortholog as well as all other samples using MEGA X (Stecher et al., 2020). The great tit is a commonly studied species for analysis of SNPs in the DRD4 exon 3 and their relation to variation in exploratory behaviour (Fidler et al., 2007; Riyahi et al., 2017; Timm et al., 2019).

The sequences were surveyed for SNPs throughout the exon, including at sites known to have prevalent polymorphisms in other species such as SNP830 (Fidler et al., 2007; Riyahi et al., 2017; Timm et al., 2019). I used electropherograms to confirm the presence of a SNP in a sample sequence, and to determine the zygosity (i.e., heterozygous or homozygous) for each individual at each variable site identified in the population. I classified each SNP as synonymous or non-synonymous by translating the sequence using MEGA X (Stecher et al., 2020) and determined whether the amino acid sequence was affected by the polymorphism. Each SNP was tested for Hardy-Weinberg equilibrium and all pairs of SNPs tested for linkage disequilibrium using the R package “Genetics” (Version 1.3.8.1.2; Warnes et al., 2019).

2.5 *Statistical analysis*

I used principal component analyses (PCA) to reduce dimensionality of the 7 behaviours scored to assess exploration. Because I was primarily interested in exploration of a novel environment, my main dataset included only the first exploration trial for each individual. No retrials were included in this analysis and for those individuals that were tested in both 2018 and 2019, only the 2018 trial was included in the analysis. Thus, each

bird's exploratory scores for this analysis represent a response to a truly novel environment. I retained the component explaining the most variation (PC1) and interpreted it as exploratory behaviour.

As a complementary analysis and in order to assess repeatability of exploratory behaviour, I conducted a second PCA including retrials as well as first trials (total of 117 trials on 80 unique birds) and again retained the component explaining the most variation. For the 37 individuals that were tested twice, I calculated repeatability of exploration scores (PC1) using the method of Lessells & Boag, (1987). This approach uses a one-way ANOVA to calculate the variance among-groups (i.e., among individuals) and within-groups (i.e., multiple measurements of the same individual), and calculates repeatability as: among-individual variance / (among-individual variance + within-individual variance).

To test for the effect of possible covariates on the relationship between the movement behaviours, three linear regression models were used with latitudinal migration distance or total migration distance as the response variable, exploration as the independent variable, and sex, age, year, tarsus length, and the day of first capture as covariates. The day of first capture was recorded as the date the bird was first caught in relation to the first day of the field season (i.e., April 15th corresponds to day 1).

To test for differences in exploratory behaviour scores, latitudinal migration distance (δ^2H), and total migration distance between individuals with and without SNPs at certain sites in the DRD4 exon 3, Welch's independent samples t-tests were used. The Welch's test was used to account for unequal variance and unequal sample sizes of

individuals with and without the SNPs. Hedge's g was used to measure effect size for each test (because of the small sample size of SNP occurrences) using the R package "effsize" (version 0.7.6; Torchiano, 2019).

The relationship between circulating androgen concentrations and the movement behaviours of focus was assessed using a linear regression including possible covariates. Androgen concentrations were not normally distributed (Shapiro-Wilk, $W_{78} = 0.693$, $p = 1.76 \times 10^{-11}$), and required \log_{10} transformation to yield normality. The three regression models included either exploration scores, latitudinal migration distance (δ^2H), or total migration distance as the dependent variable, \log_{10} transformed androgen concentrations as the independent variable, and sex, year, tarsus length, and the date each bird was bled as possible covariates. The bleed date was recorded as the day the bird was first bled in relation to the first day of blood sampling (i.e., April 15th corresponds to day 1).

For all models, Akaike information criterion model selection for small sample sizes (AICc) was used to assess model fit of all possible subsets of variables. Models with the lowest AICc value were selected, unless the $\Delta AICc$ was < 2 (as suggested in Mazerolle, 2006). If this occurred, I selected the model with the most consistently retained variables found in the other top models (those with $\Delta AICc < 2$). The 'dredge' function from the R package 'MuMIn' (version 1.43.10; Barton, 2019) was used to calculate the AICc values for all models. The models were tested to assess whether they met the required assumptions (i.e., normality, linearity, variance, leverage, and the relationship between standard residuals and independent variables). Additional R packages used included 'dplyr' (version 0.8.3; Wickham et al., 2019) for data

organization, as well as ‘ggplot2’ (version 3.2.1; Wickham, 2016) and ‘ggpubr’ (version 0.2.4; Kassambara, 2019) to visualize data. All data were analyzed with RStudio version 1.2.1578.

3. Results

3.1 Variation in exploration

All seven exploratory behaviours measured were highly correlated with each other when including all trials per individual (i.e., including retrials; Table 3.1), and almost all were highly correlated when only including first trials (i.e., excluding retrials; Table 3.2). Accordingly, to reduce dimensionality I analyzed exploratory behaviour using principal component analyses (PCA). I conducted two separate PCAs. The first analysis included all recorded trials (i.e., including retrials) and was conducted to assess repeatability of exploratory scores for the subset of individuals that were tested twice, whereas the second excluded retrials and thus represents each individual's first response to the test, i.e., to a truly novel environment.

For both analyses, only the first principal component had an eigenvalue greater than one, and PC1 accounted for approximately half of the variation in exploratory behaviour (Tables 3.3 and 3.4 respectively for analyses including and excluding retrials). Thus, for both PCAs I retained only PC1 for further analysis. PC1 was not normally distributed in the analyses with retrials (Shapiro-Wilk, $W_{130} = 0.950$, $p = 1.17 \times 10^{-4}$), but was normally distributed when excluding retrials (Shapiro-Wilk, $W_{78} = 0.975$, $p = 0.129$). High positive values of PC1 in both analyses were associated with high numbers of flights, trees visited, features visited, and sectors visited, and with shorter latency to visit the first new feature. The PC1 score for one individual's first ever trial was determined to be an outlier as it was over three times more than the interquartile range above the third quartile, and 3.64 standard deviations above the mean of all PC1 scores. This individual was removed from further analyses including exploratory behaviour. Among the 37 birds

Table 3.1. Spearman correlation matrix of exploratory behaviours for all trials.

	Flights	Hops	Sectors Visited	Trees Visited	Features Visited	Head Scanning Rate	Latency to move
Flights		0.439	0.708	0.738	0.841	0.464	-0.468
Hops			0.481	0.581	0.676	0.273	-0.232
Sectors Visited				0.661	0.729	0.331	-0.262
Trees Visited					0.845	0.375	-0.299
Features Visited						0.426	-0.452
Head Scanning Rate							-0.214
Latency to Move							

Note: Song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. Both first and retrials were included (N = 117 tests on 80 individual birds). All pairwise combinations of behaviours were significantly correlated at $\alpha = 0.05$.

Table 3.2. Spearman correlation matrix of exploratory behaviours for first ever trials.

	Flights	Hops	Sectors Visited	Trees Visited	Features Visited	Head Scanning Rate	Latency to move
Flights		0.277	0.716	0.686	0.792	0.420	-0.434
Hops			0.332	0.456	0.550	0.278	-0.082
Sectors Visited				0.638	0.700	0.346	-0.281
Trees Visited					0.811	0.317	-0.240
Features Visited						0.397	-0.370
Head Scanning Rate							-0.216
Latency to Move							

Note: Song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. Only first ever trials (N = 80 birds) were included. Correlation coefficients shown in bold were significantly correlated at $\alpha = 0.05$.

Table 3.3. Loading scores for principal component analysis (PCA) of exploratory behaviours for all trials.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Flights	0.376	0.300	-0.489	0.156	-0.463	-0.513	0.170
Hops	0.288	0.251	0.818	0.289	-0.271	-0.034	0.165
Sectors Visited	0.413	0.030	0.090	0.008	0.767	-0.477	-0.064
Trees Visited	0.467	0.084	-0.207	-0.036	0.190	0.599	0.580
Features Visited	0.489	0.118	-0.086	0.044	-0.101	0.357	-0.774
Head Scanning Rate	0.221	-0.840	-0.035	0.479	-0.109	-0.040	0.038
Latency to Move	-0.314	0.346	-0.182	0.812	0.256	0.146	-0.046
Eigenvalue	3.581	0.927	0.813	0.708	0.523	0.318	0.129
Cumulative variance explained (%)	51.161	13.247	11.616	10.119	7.469	4.549	1.839

Note: Song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. Both first and retrials were included (N = 117 tests on 80 individual birds).

Table 3.4. Loading scores for principal component analysis (PCA) of exploratory behaviours for first ever trials.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Flights	0.392	0.435	-0.210	0.207	-0.426	0.610	-0.132
Hops	0.179	-0.819	-0.446	0.180	-0.209	0.082	-0.127
Sectors Visited	0.427	-0.072	-0.034	0.009	0.825	0.354	0.072
Trees Visited	0.466	0.207	-0.145	0.051	0.076	-0.590	-0.601
Features Visited	0.488	0.060	-0.086	0.091	-0.162	-0.364	0.764
Head Scanning Rate	0.251	-0.245	0.819	0.420	-0.129	0.039	-0.107
Latency to Move	-0.338	0.170	-0.239	0.859	0.213	-0.114	0.065
Eigenvalue	3.486	0.993	0.883	0.673	0.466	0.330	0.170
Cumulative variance explained (%)	49.794	14.184	12.612	9.609	6.654	4.712	2.435

Note: Song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. Only first trials were included (N = 80 birds).

that were tested twice, individual's exploration scores (PC1) derived from the first PCA (i.e., including retrials) were not significantly repeatable between the first and second trials (repeatability = 0.188; ANOVA, $F_{1,36} = 1.479$, $p = 0.121$; Table 3.5).

3.2 *Variation in migration distance*

Two measures of migration distance were assessed for their relationship with exploratory behaviour. Latitudinal migration distance (mean \pm SE = -53.13 ± 1.06 ‰, range = $-78.42 - -30.47$ ‰) was based on the stable isotope values ($\delta^2\text{H}$) of each individual's claw sample, inferring overwintering latitude. This variable was normally distributed (Shapiro-Wilk, $W_{78} = 0.987$, $p = 0.631$).

Total migration distance (mean \pm SE = 1559.4 ± 14.68 km, range = $883.2 - 1736.7$ km) was estimated based on the distance of the centroid of each individual's likely overwintering range from the breeding site (Figure 3.1). Latitudinal migration distance and total migration distance were significantly correlated (Spearman's rank, $r_{77} = 0.972$, $p = 2.2 \times 10^{-16}$, Figure 3.2). However, three datapoints (the leftmost three points in Figure 3.2, corresponding to the three most northerly likely overwintering ranges and the shortest three total migration distances) were identified as statistical outliers in this analysis as they were over three times greater than the interquartile range above the third quartile, and greater than three standard deviations above the mean of all distances. The poorer fit of these most northerly points to the isoscape likely reflect lake effects and wider isoscape bands at these latitudes, resulting in more uncertainty in the calculation of the three most northerly centroids. In the interest of being conservative, analyses involving total migration distance present results both including and excluding these

Table 3.5. Repeatability of exploratory scores (PC1) between first and second trials.

Source of Variation	<i>df</i>	Sum of Squares	Mean Squares	<i>F</i>	<i>P</i>
Among individuals	36	166.6	4.628	1.479	0.121
Within individuals	37	115.8	3.130		

Note: Song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019 (N = 74 tests on 37 individuals).

Figure 3.1. Likely overwintering origins of song sparrows based on $\delta^2\text{H}$ stable isotope values of claw samples.

Likely overwintering origins of 78 migratory song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019 based on $\delta^2\text{H}$ stable isotope values of claw samples. The legend shows the number of individuals assigned to a pixel based on the odds from a multivariate normal likelihood approach (Van Wilgenburg & Hobson, 2011). Symbols (+) represent the centroid of each individual song sparrow's likely overwintering range. Overall likelihood model was framed using song sparrow range data from BirdLife International (2019).

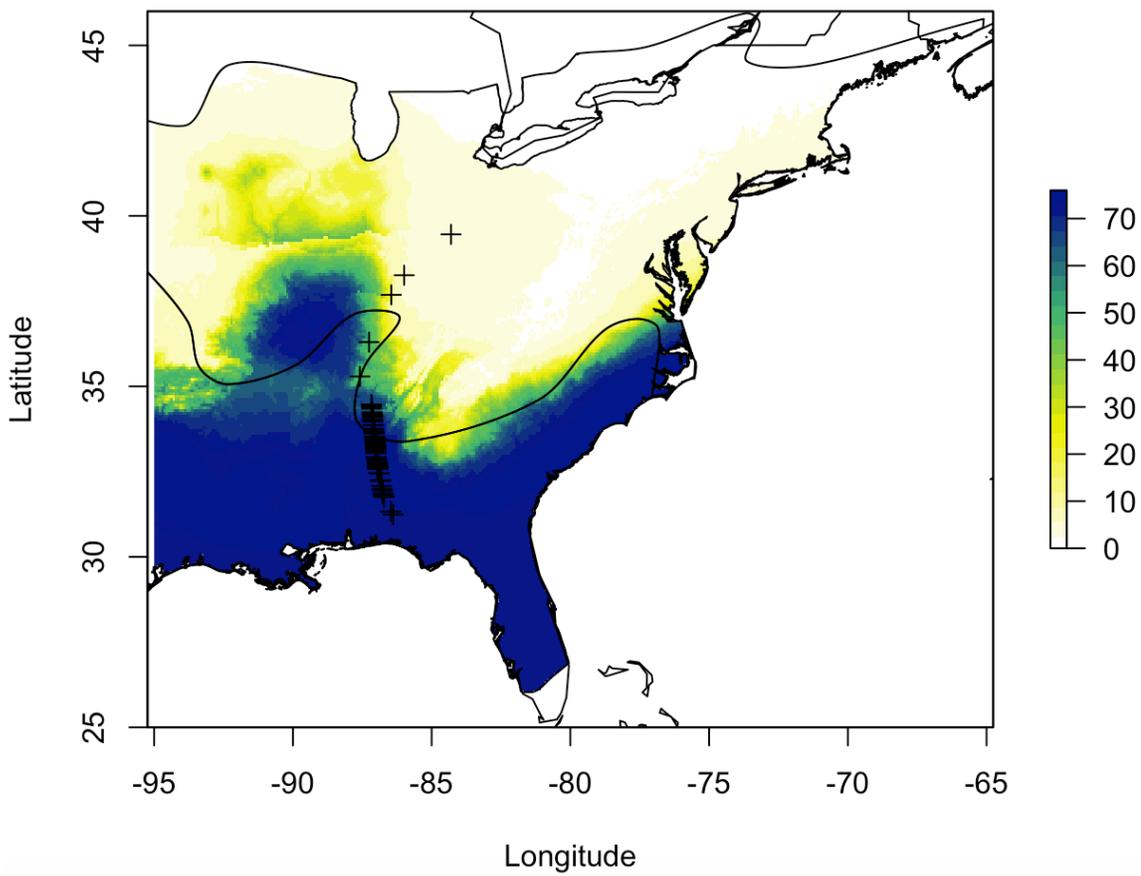
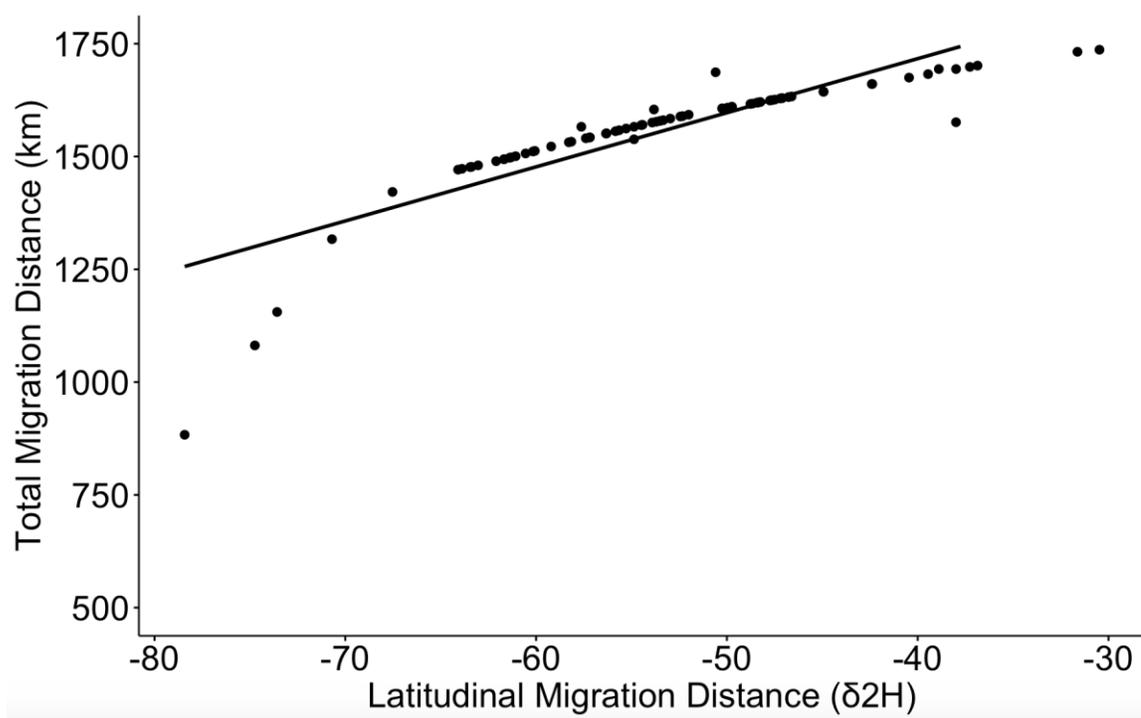


Figure 3.2. Correlation between latitudinal migration distance and total migration distance.

Correlation between latitudinal migration distance (inferred from $\delta^2\text{H}$ values; less negative values indicate more southerly overwinter location and thus longer latitudinal distance) and total migration distance (inferred from likely overwintering origins) of 78 migratory song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. One measurement of each individual's migration distance was analyzed (values derived from first claw collection) for each metric. The two measurements of migration distance show a strong positive relationship, although fit is poorer at extreme northerly values (leftmost three points). The solid line represents the least-squares regression fitted for illustrative purposes.



three northernmost data points. Total migration distance including all data points was not normally distributed (Shapiro-Wilk, $W_{78} = 0.734$, $p = 1.41 \times 10^{-10}$) and this could not be remedied by transformation. However, total migration distance excluding the three northernmost data points was normally distributed (Shapiro-Wilk, $W_{78} = 0.976$, $p = 0.175$). Accordingly, correlation tests involving total migration distance including all data points used Spearman's rank correlation while those excluding the three northernmost points used Pearson's correlation.

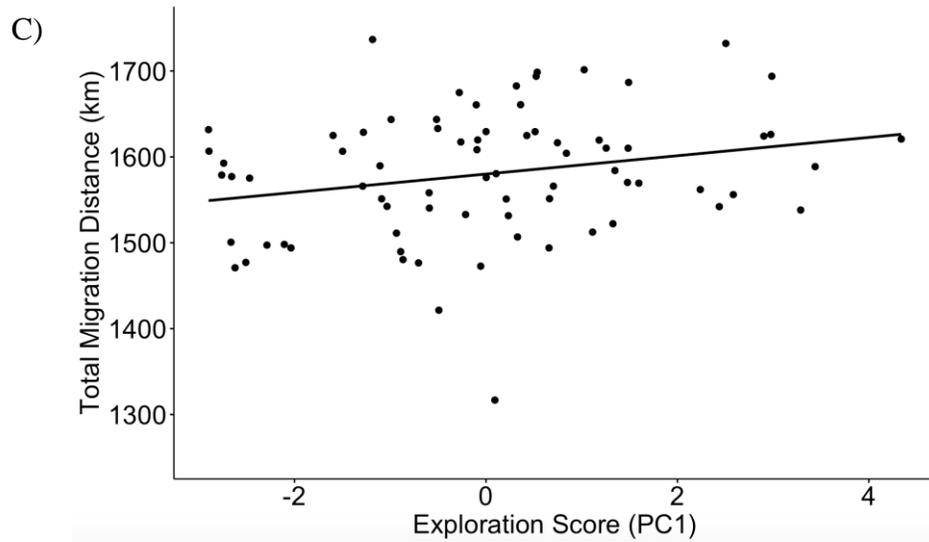
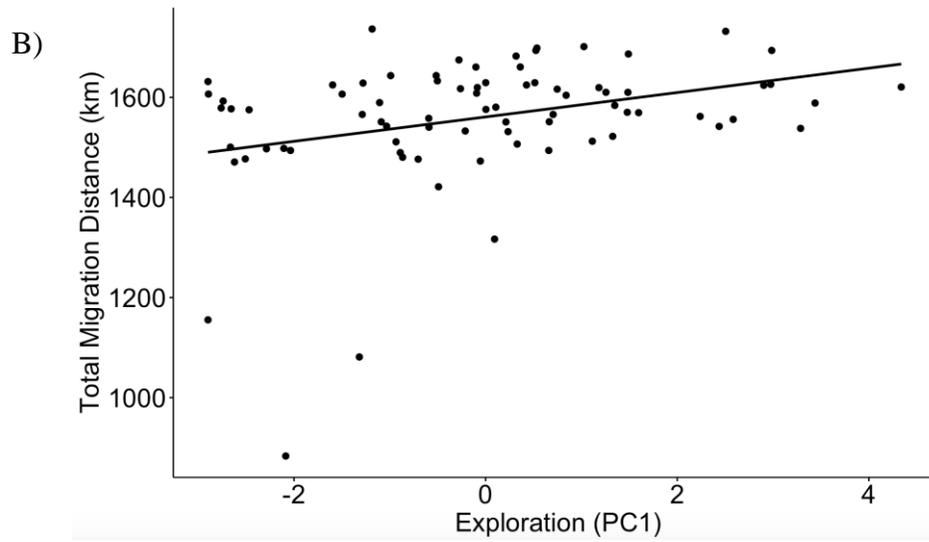
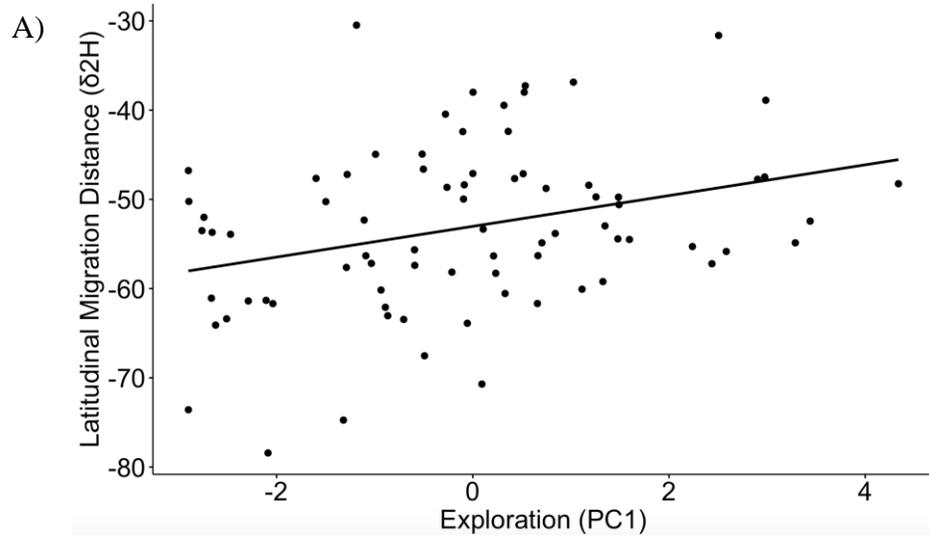
3.3 *Relationship between exploration and migration*

PC1 scores of the first ever exploratory trials (hereafter referred to as 'exploration scores') were positively related to estimates of latitudinal migration distance (Pearson's $r_{77} = 0.316$, $p = 0.005$, Figure 3.3A). PC1 scores were also positively related to total migration distance, regardless of whether the full dataset was used or the three northernmost values were excluded (full dataset: Spearman's $r_{77} = 0.292$, $p = 0.009$, Figure 3.3B; excluding northernmost values: Spearman's $r_{74} = 0.241$, $p = 0.037$, Figure 3.3C).

As a follow-up to these analyses, I conducted linear regressions to correct for potential effects of covariates including age class, sex, year, date of first capture, and tarsus length. AICc model selection identified the best-supported model predicting latitudinal migration distance as including exploration score, sex, and date of first capture (Table 3.6; model selection table in appendix, Table A.1). Specifically, latitudinal migration distance varied positively with exploration scores (i.e., birds that traveled farther south had higher exploration scores), was greater for females than for males (i.e.,

Figure 3.3. Correlation between exploration scores and latitudinal migration distance or total migration distance.

Correlation between exploration scores (PC1) and (A) latitudinal migration distance (inferred from $\delta^2\text{H}$ values; less negative values indicate more southerly overwinter location and thus longer latitudinal distance), (B) total migration distance (inferred from likely overwintering origins), (C) total migration distance excluding the three northernmost values in 78 song sparrows (*Melospiza melodia*) and caught near Newboro, Ontario in April-May 2018 and 2019. Both latitudinal and total migration distances (regardless of whether northernmost values were included) were positively associated with exploration score. The solid line represents the least-squares regression fitted for illustrative purposes.



females overwintered farther south), and varied negatively with date of first capture (i.e., birds that traveled farther south were captured earlier in the season; Figure 3.4A). For total migration distance using the full dataset, the best-supported model again included exploration score, which varied positively with total migration distance, and date of first capture, which varied negatively with total migration distance; however total migration distance using the full dataset did not differ significantly between the sexes (Figure 3.4B, Table 3.7; model selection table in appendix, Table A.2). Analysis of total migration distance excluding the three northernmost values aligned with the pattern seen for latitudinal migration distance. That is, the best supported model included only sex (females migrated farther than males) and date of first capture (birds that traveled farther were captured earlier in the season; Figure 3.4C; Table 3.7; model selection table in appendix, Table A.3).

3.4 Plasma androgen concentrations and movement behaviours

3.4.1 Androgens and exploration

AICc selection of models predicting exploratory scores identified the best model as containing only date of blood sampling as a predictor (Table 3.8; AICc table of models in appendix, Table A.4). That is, androgen concentrations were eliminated from the model predicting exploration scores; other excluded variables were sex, year, and

Table 3.6. Linear regression model of latitudinal migration distance and exploratory scores.

Dependent Variable	Independent Variable	<i>T</i>	<i>df</i>	95% CI	<i>P</i>
Latitudinal migration distance ($\delta^2\text{H}$)	Exploration Score (PC1)	2.311	3, 74	0.179, 2.420	0.024
	Sex (Male)	-2.083	3, 74	-7.751, -0.172	0.041
	First Capture Day	-3.433	3, 74	-0.846, -0.225	0.001
Eliminated Variables:	Age class, Year				

Note: Model of latitudinal migration distance (inferred from $\delta^2\text{H}$) distance as a function of exploratory score (PC1), sex, date of first capture, year, and tarsus length in 78 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. The most parsimonious model is reported. Values shown in bold were significantly predictive at $\alpha = 0.05$. Multiple R-squared = 0.247, adjusted R-squared = 0.217.

Table 3.7. Linear regression models of total migration distance including and excluding three northernmost values and exploratory scores.

Dependent Variable	Independent Variable	<i>T</i>	<i>df</i>	95% CI	<i>P</i>
Total migration distance (including northernmost values)	Exploration Score (PC1)	2.625	2, 75	5.014, 36.575	0.011
	First Capture Day	-2.985	2, 75	-10.938, -2.182	0.004
Eliminated Variables:		Sex, Age class, Year			
Total migration distance (excluding northernmost values)	Sex (Male)	-2.616	2, 72	-75.323, -10.177	0.011
	First Capture Day	-2.215	2, 72	-5.795, -0.305	0.0299
Eliminated Variables:		Sex, Age class, Year			

Note: Models of total migration distance including and excluding three northernmost values as a function of exploratory score (PC1), sex, date of first capture, year, and tarsus length in 78 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. The most parsimonious models are reported. Values shown in bold were significantly predictive at $\alpha = 0.05$. Model including northernmost values: multiple R-squared = 0.199, adjusted R-squared = 0.177. Model excluding northernmost values: multiple R-squared = 0.132, adjusted R-squared = 0.108.

Figure 3.4. Linear regression of first day of capture as a function of latitudinal migration distance or total migration distance.

Linear regression of first day of capture (days since the first date of the field season, April 15th) as a function of (A) latitudinal migration distance (inferred from $\delta^2\text{H}$ values; less negative values indicate more southerly overwinter location and thus longer latitudinal distance), (B) total migration distance including the three northernmost values, and (C) total migration distance excluding the three northernmost values, of 78 migratory song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. Date of first capture decreased with both latitudinal and total migration distance (i.e., birds traveling longer distances were first captured earlier in the spring).

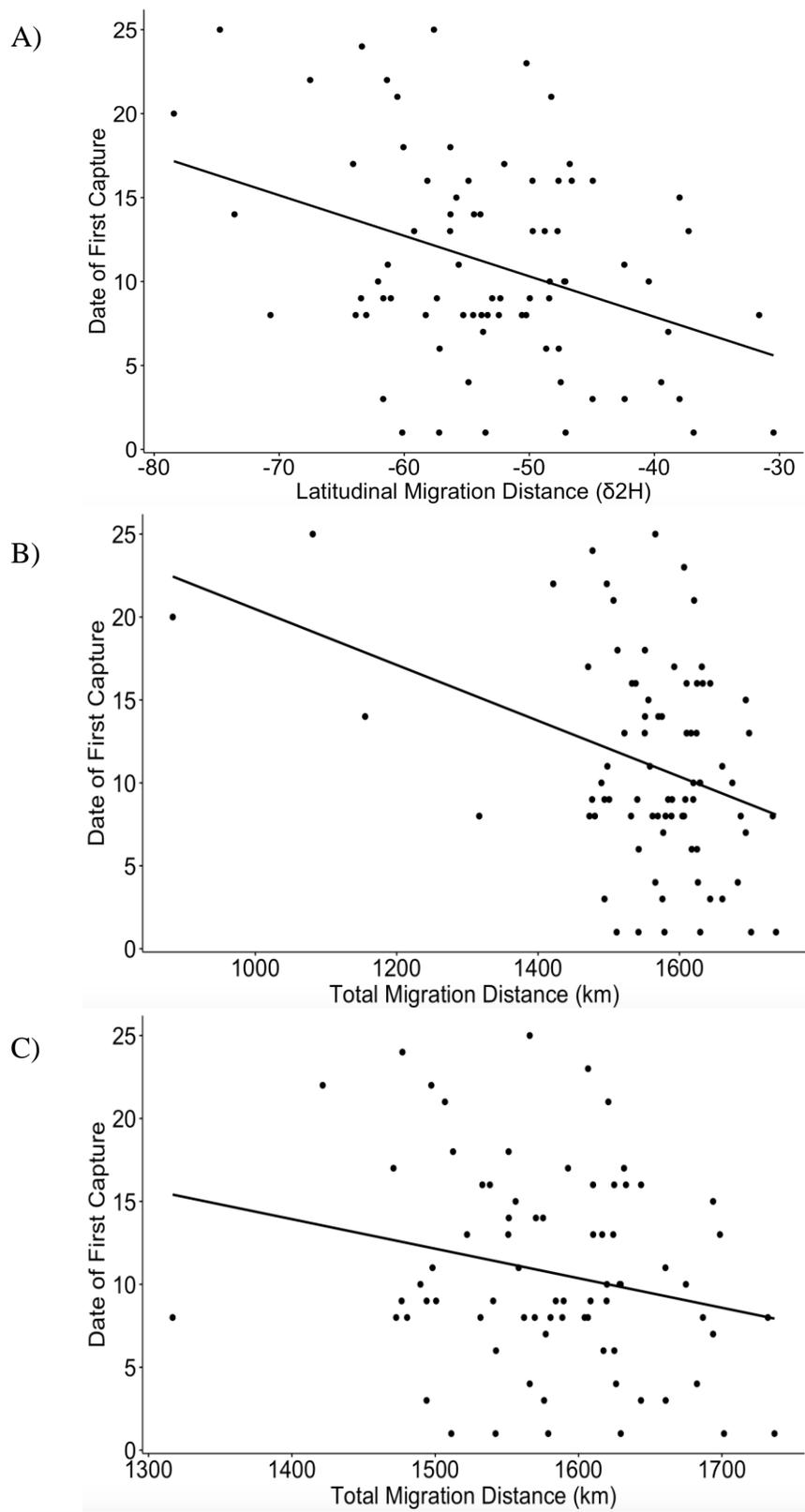


Table 3.8. Linear regression model of exploration scores (PC1) and logarithmically transformed androgen concentrations.

Dependent Variable	Independent Variable	<i>T</i>	<i>df</i>	95% CI	<i>P</i>
Exploration Score (PC1)	Date of blood sampling	-2.267	1, 76	-0.129, -0.008	0.026

Eliminated Variables: Sex, Year, Age, log[Androgens], Tarsus

Note: Regression model of exploration scores (PC1) as a function of logarithmically transformed androgen concentration, date of blood sampling, sex, year, and tarsus length of 78 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. The most parsimonious model is reported. Values shown in bold were significantly correlated at $\alpha = 0.05$. Multiple R-squared = 0.063, adjusted R-squared = 0.051.

tarsus length. Exploration scores decreased with date of blood sampling, such that birds sampled later in the season were less exploratory (linear regression, $t_{1,75} = -2.267$, $p = 0.026$, Figure 3.5A).

Most birds had their exploration trials conducted on the same day that blood was collected, or separated by just a few days such that androgen levels at blood collection were presumably similar to those at the time of behavioural testing. However, this was not always the case, and long intervals between blood sampling and behavioural testing might obscure any effect of androgen concentrations on behaviour. Accordingly, I identified the subset of individuals ($n = 69$) that had blood collected (for androgen analysis) and behavioural trials conducted no more than five days apart. For this subset of birds with blood collection and behavioural tests closely linked in time, the best model predicting exploratory scores was the null model (AICc table of models in appendix, Table A.5). That is, androgen concentrations were not predictive of exploration scores; other excluded variables were date of blood sampling, sex, year, and tarsus.

3.4.2 *Androgens and migration distance*

AICc model selection of models predicting latitudinal migration distance identified the best model as containing date of blood sampling and sex as predictors (Table 3.9; AICc table of models in appendix, Table A.6). That is, androgen concentrations were eliminated from the model predicting latitudinal migration distance; other excluded variables were year and tarsus length. Latitudinal migration distance decreased with date of blood sampling, such that birds sampled earlier in the season had less negative claw $\delta^2\text{H}$ values and were presumably returning from longer latitudinal

Figure 3.5. Linear regression of movement behaviours as a function of the date of blood sampling.

Movement behaviours as a function of the date of blood sampling (days since the date of first blood sampling, April 15th) for 78 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. (A) Exploration scores (PC1), (B) latitudinal migration distance (inferred from $\delta^2\text{H}$ values), (C) total migration distance (inferred from likely overwintering origins) over the full dataset, and (D) total migration distance excluding the three northernmost values, all decreased with increasing date of blood sampling (days since blood sampling, with April 15th corresponding to day 1).

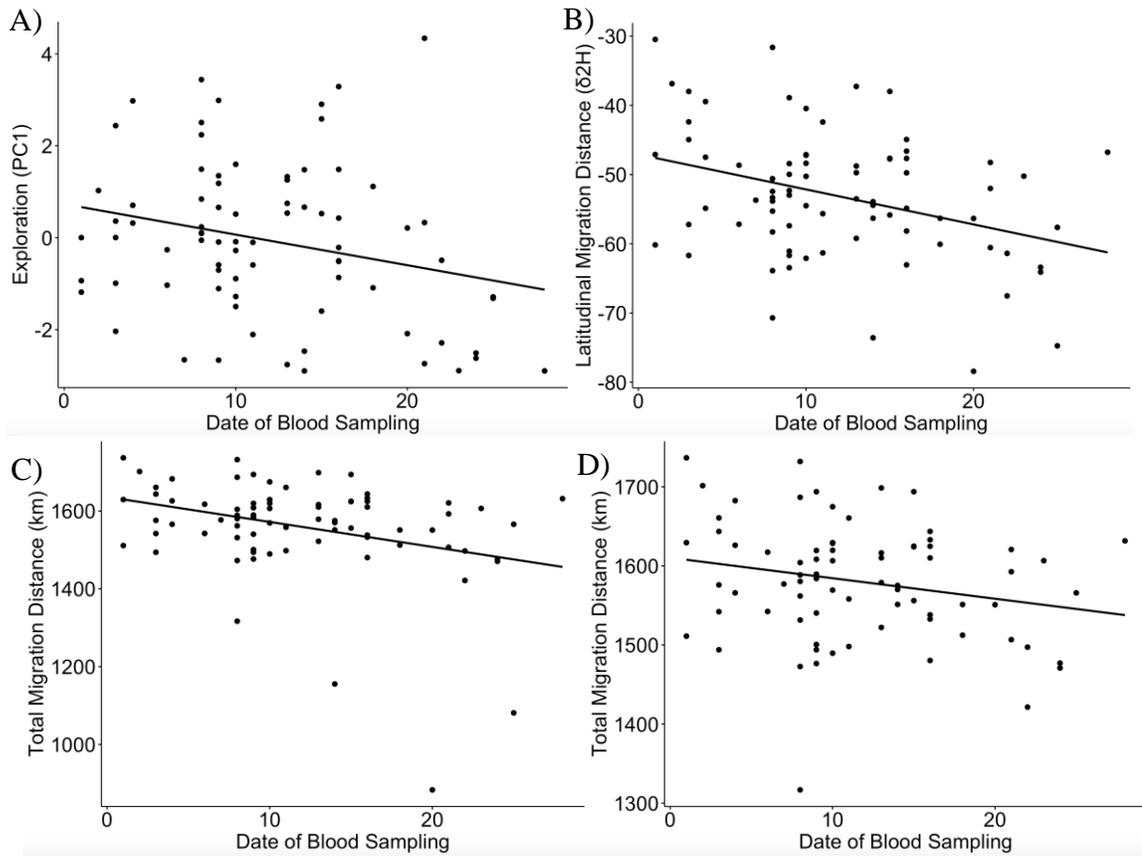


Table 3.9. Linear regression models of latitudinal migration distance or total migration distance including or excluding the three northernmost values and logarithmically transformed androgen concentration.

Dependent Variable	Independent Variable	<i>T</i>	<i>df</i>	95% CI	<i>P</i>
Stable Isotope Value ($\delta^2\text{H}$) (latitudinal migration distance)	Sex (Male)	-2.314	2, 75	-8.411, -0.629	0.023
	Date of blood sampling	-3.589	2, 75	-0.847, -0.242	5.89x10⁻⁴
Eliminated Variables:	Year, Age, log[Androgen], Tarsus				
Total Migration Distance (including northernmost values)	Date of blood sampling	-6.419	1, 76	-10.753, -2.085	0.004
	Eliminated Variables:	Sex, Year, Age, log[Androgens], Tarsus			
Total Migration Distance (excluding northernmost values)	Date of blood sampling	-2.201	2, 72	-5.439, -0.270	0.031
	Sex (Male)	-2.622	2, 72	-75.473, -10.282	0.011
Eliminated Variables:	Year, Age, log[Androgen], Tarsus				

Note: Models of latitudinal migration distance (inferred from $\delta^2\text{H}$) or total migration distance as functions of logarithmically transformed androgen concentration, date of blood sampling, sex, year, and tarsus length of 78 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. The most parsimonious model is reported. Bold type denotes predictors that were significantly predictive at $\alpha = 0.05$. Latitudinal migration distance: multiple R-squared = 0.181, adjusted R-squared = 0.159. Total migration distance including northernmost values: multiple R-squared = 0.103, adjusted R-squared = 0.091. Total migration distance including northernmost values: multiple R-squared = 0.131, adjusted R-squared = 0.107.

migrations (linear regression, $t_{2,75} = -3.589$, $p = 5.89 \times 10^{-4}$, Figure 3.5B). Females also had less negative claw $\delta^2\text{H}$ values and thus had presumably returned from longer latitudinal migrations than males (linear regression, $t_{2,75} = -2.314$, $p = 0.023$).

When using total migration distance as the dependent variable and including all datapoints, AICc model selection identified the best model as containing only date of blood sampling as a predictor (Table 3.9; AICc table of models in appendix, Table A.7). That is, androgen concentrations were eliminated from the model predicting total migration distance; other excluded variables were sex, year, and tarsus length. Total migration distance decreased with date of blood sampling, such that birds sampled earlier in the season appeared to be returning from longer migrations (linear regression, $t_{1,76} = -6.419$, $p = 0.004$, Figure 3.5C).

When excluding the three northernmost values using total migration distance as the dependent variable, results were generally similar except that sex as well as date of blood sampling was identified as a predictor (Table 3.9; AICc table of models in appendix, Table A.8). Similar to the whole-dataset analysis, androgen concentrations did not appear predictive of total migration distance and birds sampled earlier in the season appeared to be returning from longer migrations (linear regression, $t_{2,72} = -2.064$, $p = 0.043$, Figure 3.5D). Moreover, females also had greater total migration distances and thus had presumably returned from longer latitudinal migrations than males (linear regression, $t_{2,72} = 2.622$, $p = 0.011$) for this subset of individuals.

3.5 *DRD4* variation and association with movement behaviours

3.5.1 *Genetic variation at DRD4 exon 3*

I identified the consensus sequence for *DRD4* exon 3 in song sparrows, based on the sequences retrieved from 77 individuals. The sequenced region was 548 bp in length, corresponding to amino acids 115 through 297 in the *DRD4* gene, and quite similar to the great tit orthologue to which the song sparrow sequences were initially aligned (96.2% nucleotide similarity, 96.7% amino acid similarity; Figure 3.6). The two species differed by 18 base pairs in their *DRD4* exon 3 sequences, 12 reflecting synonymous substitutions, and 6 reflecting five non-synonymous substitutions (two nucleotide differences belonged to the same codon). In addition, there was an in-frame insertion (3 bp) in great tit relative to song sparrow. SNP positions are reported with reference to the start of the *DRD4* gene, excluding intronic regions (following Fidler et al., 2007).

Among the song sparrows sequenced, I identified 6 different SNPs (Table 3.10), occurring in a total of seventeen birds. No birds had more than one SNP, and in each case the minor allele was heterozygous rather than homozygous. Estimates of linkage disequilibrium were close to zero for all pairwise combinations of SNPs (all $D < 0.01$), and each SNP was in Hardy-Weinberg equilibrium ($p > 0.9$ for all loci). I selected the most common three SNPs (C734T, $n = 4$; A742G, $n = 5$; C827T, $n = 4$) to test for associations with movement behaviours. C827T, a synonymous SNP, corresponds to a SNP associated with variation in novelty seeking behaviours in great tits (SNP C830T; Fidler et al., 2007; Timm et al., 2019). C734T also reflects a synonymous variant, while A742G reflects a non-synonymous variant resulting in an amino acid substitution (lysine-to-serine). Because of the low prevalence of SNPs observed within the birds sequenced, I

Figure 3.6. Great Tit and Song Sparrow protein alignment

Protein alignment for exon 3 of the DRD4 gene for the Song Sparrow (*Melospiza melodia*) relative to the Great Tit (*Parus major*) orthologue. Black squares indicate matching amino acids in the Song Sparrow relative to the Great Tit. Grey squares indicate different amino acids that have the same charge. White squares indicate differing amino acids between the two species.

GreatTit	1	FIAVQIPLNYNRRQIDLRQLILISTTWIFAFAVASPVIFGLNNVPNRDPS
SongSparrow	1	FIAVQIPLNYNRRQIDLRQLILISTTWIFAFAVASPVIFGLNNVPCRDRPS
GreatTit	51	LCQLEDDNYIVYSSICSFIPCPVMLVLYCGMFQGLKRWEEARKAKLRGC
SongSparrow	51	LCQLEDDNYIVYSSICSFIPCPVMLVLYCGMFQGLKRWEEARKAKLRGC
GreatTit	101	IYGANRKLYHPPTLMEREQTRLGLLD CSSPYARAGLPGECGMNSGIQTVS
SongSparrow	101	IHGANKLYHPPTLMEREQTRLGLLE C-SPYARAGLPGECGMNSGIQTVS
GreatTit	151	YPHLRYPHPGHGHRKRAKINGRERKAMRVLPVVV
SongSparrow	150	YPHLRYPHPGHGRKQAKINGRERKAMRVLPVVV

Table 3.10. Single nucleotide polymorphisms (SNPs) identified in exon 3 of DRD4.

SNP	Amino Acid Change	Substitution Type	Amino Acid Group Change	Proportion (%Minor Alleles)
C734T	217His→His	Synonymous	None	4/77 (2.6%)
A742G	220Lys→Ser	Non-Synonymous	Polar+ → Polar	5/77 (3.3%)
G791A	236Arg→Arg	Synonymous	None	2/77 (1.3%)
C827T	248Ala→Ala	Synonymous	None	4/77 (2.6%)
C908T	275His→His	Synonymous	None	1/77 (0.6%)
C932T	283Asn→Asn	Synonymous	None	1/77 (0.6%)

Note: SNPs identified in exon 3 of DRD4 in 77 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario. A total of 17 individuals had variant sequences (only one SNP per individual and heterozygous in each case). SNP A742G causes a non-synonymous amino acid change from lysine, which has a polar-positive side chain, to serine, which has a polar-uncharged side chain. Synonymous SNP C827T is equivalent to synonymous SNP C830T in great tits (*Parus major*) that has been associated with differences in novelty seeking behaviours (Fidler et al., 2007; Timm et al., 2019).

also tested whether having any SNP within this region predicted variation in exploration scores or latitudinal and total migration distance (i.e., compared movement behaviours of birds with any SNP versus birds with no SNPs; Table 3.11).

3.5.2 *DRD4* variation and movement behaviours

Song sparrows bearing the minor allele at C734T had significantly higher exploratory scores on average than those without the minor allele (Welch's $t_{8,5} = 2.681$, $p = 0.026$), but did not differ significantly in either measure of migration distance (latitudinal migration distance: Welch's $t_{3,4} = -0.003$, $p = 0.998$; total migration distance: Welch's $t_{4,1} = 0.354$, $p = 0.740$; total migration distance excluding three northernmost values: Welch's $t_{3,4} = -0.199$, $p = 0.854$; Figure 3.7). Individuals bearing the minor allele at A742G did not differ significantly from those lacking the minor allele in exploratory score (Welch's $t_{4,6} = 0.455$, $p = 0.670$), or in either measure of migration distance (latitudinal migration distance: Welch's $t_{4,2} = -0.419$, $p = 0.696$; total migration distance: Welch's $t_{4,1} = -0.759$, $p = 0.489$; total migration distance excluding three northernmost values: Welch's $t_{3,3} = 0.471$, $p = 0.667$; Figure 3.8). Individuals bearing the minor allele at C827T did not differ significantly from those lacking the minor allele in exploratory score (Welch's $t_{3,1} = 0.597$, $p = 0.591$) or in latitudinal migration distance (Welch's $t_{3,4} = 2.273$, $p = 0.097$), but had greater total migration distance than their counterparts without the minor allele (Welch's $t_{4,8} = 2.837$, $p = 0.039$) when the full dataset was used. However, this difference was not robust to excluding the three northernmost values (Welch's $t_{3,45} = 2.355$, $p = 0.087$, Figure 3.9). When considering all 6 SNPs together, individuals bearing a SNP did not differ significantly from those with no SNPs in any of

Table 3.11. Single nucleotide polymorphisms (SNPs) in exon 3 of the DRD4 gene, analyzed in association with movement behaviours.

SNP	Substitution Type	Exploration p-value (Effect Size)	Latitudinal Migration p-value (Effect Size)	Total Migration p- value (Effect Size)	Total Migration (excluding northernmost values) p-value (Effect Size)
C734T	Synonymous	0.026 (0.493)	0.998 (-0.001)	0.740 (0.109)	0.854 (-0.101)
A742G	Non-Synonymous	0.670 (0.209)	0.696 (-0.300)	0.489 (-0.859)	0.667 (0.246)
C827T	Synonymous	0.591 (0.505)	0.097 (1.043)	0.039 (0.735)	0.087 (0.994)
Any SNP		0.569 (0.162)	0.703 (0.116)	0.865 (-0.061)	0.376 (0.242)

Note: Movement behaviours of song sparrows (*Melospiza melodia*) having any SNP were also compared to those with no SNPs. Bold type indicates significant difference (at $\alpha = 0.05$) between individuals having or not having the SNP.

Figure 3.7. Occurrence of C734T within exon 3 of the DRD4 gene in relation to exploration score and latitudinal migration distance or total migration distance.

C734T occurrence in relation to exploration score (PC1) and (A) latitudinal migration distance (inferred from $\delta^2\text{H}$ values; less negative values indicate more southerly overwinter location and thus longer latitudinal distance); (B) total migration distance (inferred from likely overwintering origins) over the full dataset, (C) total migration distance excluding three northernmost values. Data from 77 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. Individuals bearing a minor allele at C734T had higher exploration scores than those without the minor allele but did not differ in latitudinal migration distance or total migration distance.

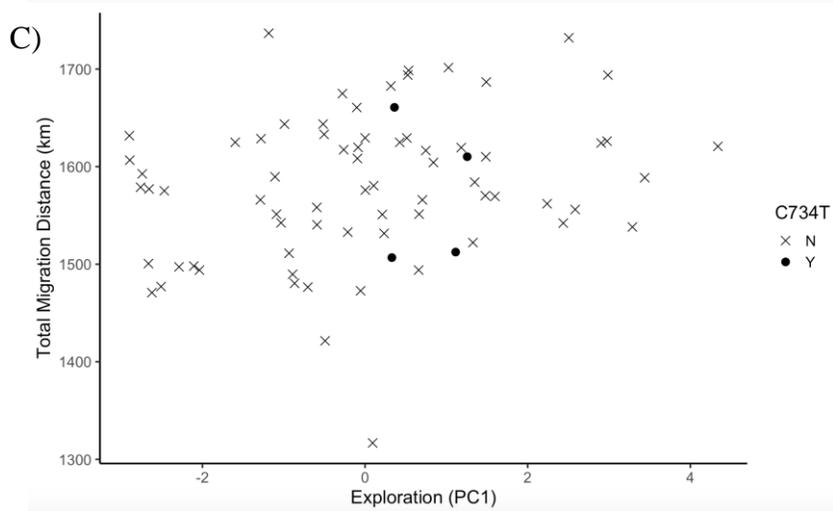
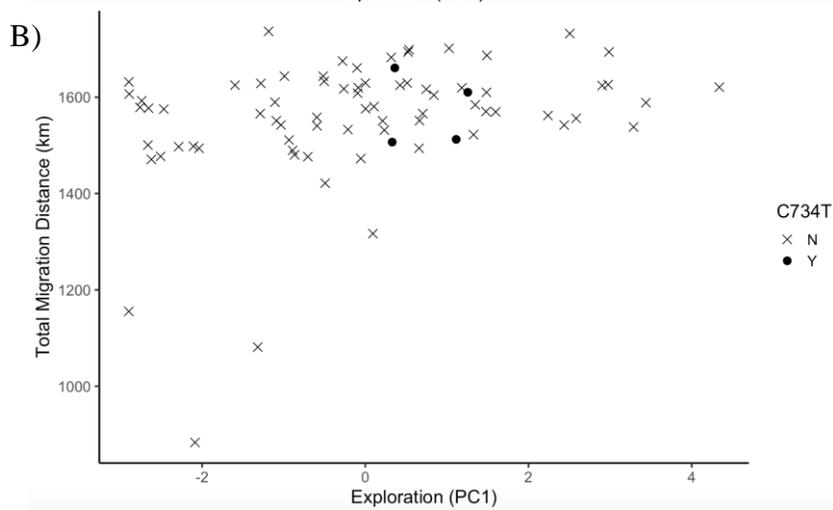
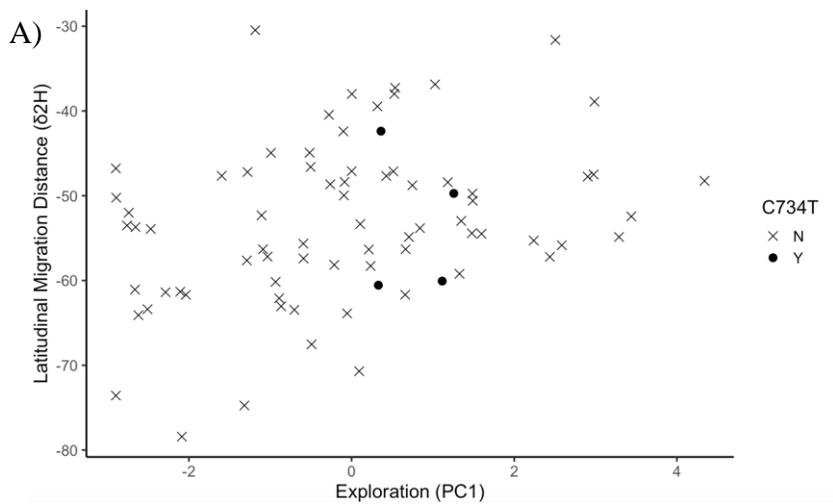


Figure 3.8. Occurrence of A742G within exon 3 of the DRD4 gene in relation to exploration score and latitudinal migration distance or total migration distance.

A742G occurrence within exon 3 of the DRD4 gene in relation to exploration score (PC1) and (A) latitudinal migration distance (inferred from $\delta^2\text{H}$ values; less negative values indicate more southerly overwinter location and thus longer latitudinal distance) or (B) total migration distance (inferred from likely overwintering origins), (C) total migration distance excluding three northernmost values. Data from 77 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. No difference was found in exploration scores, latitudinal migration distance or total migration distance between individuals with and without the minor allele at A742G.

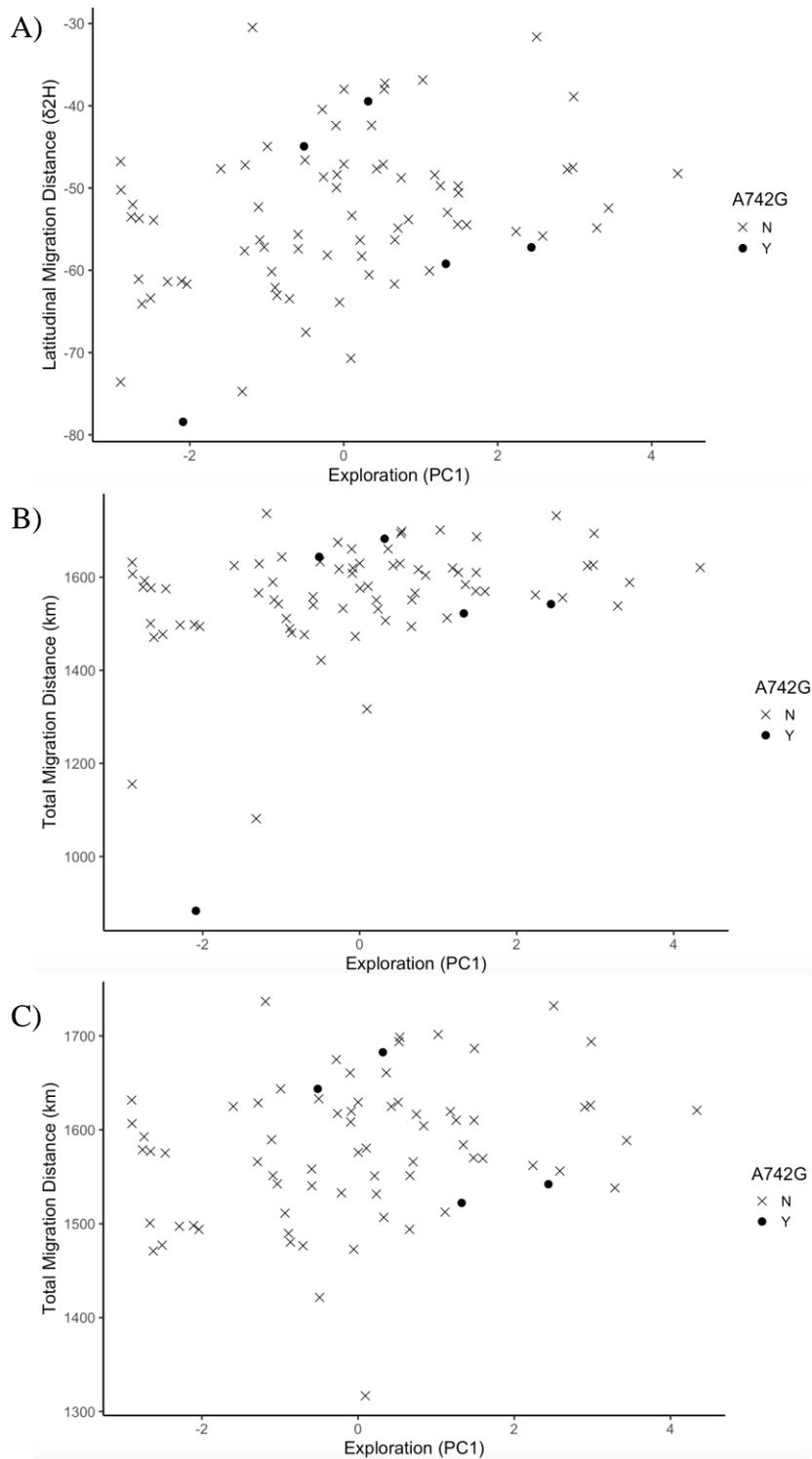
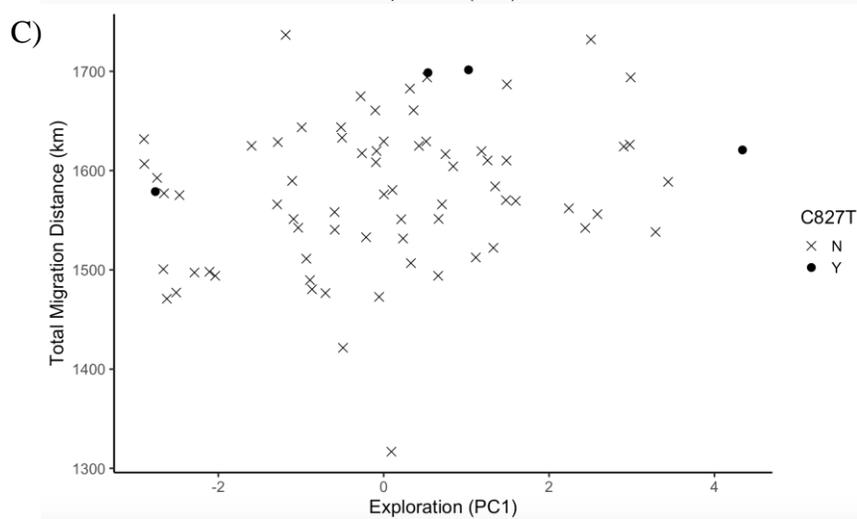
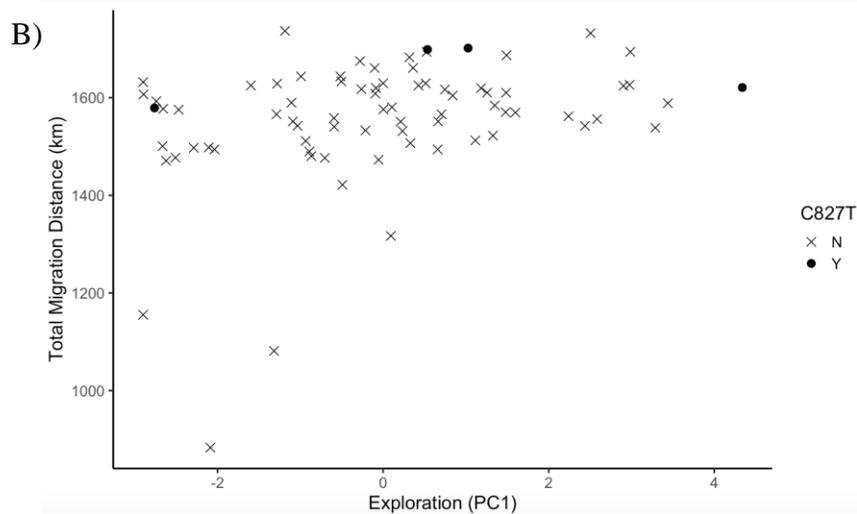
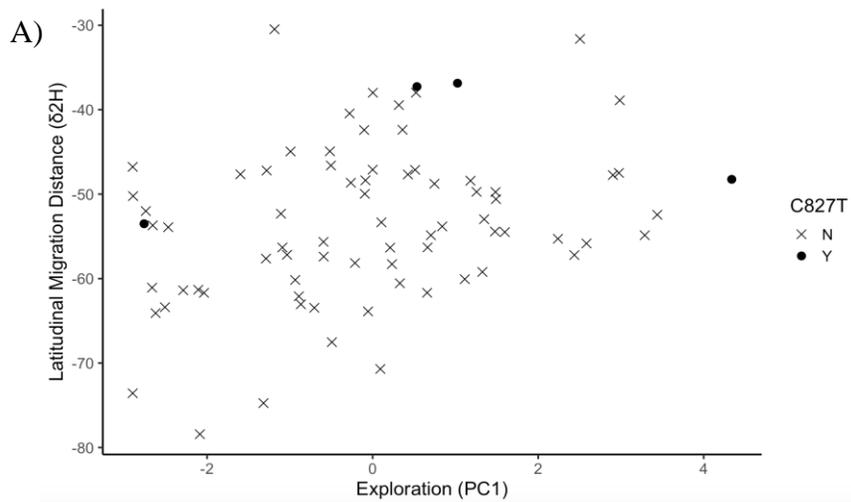


Figure 3.9. Occurrence of C827T within exon 3 of the DRD4 gene in relation to exploration score and latitudinal migration distance or total migration distance.

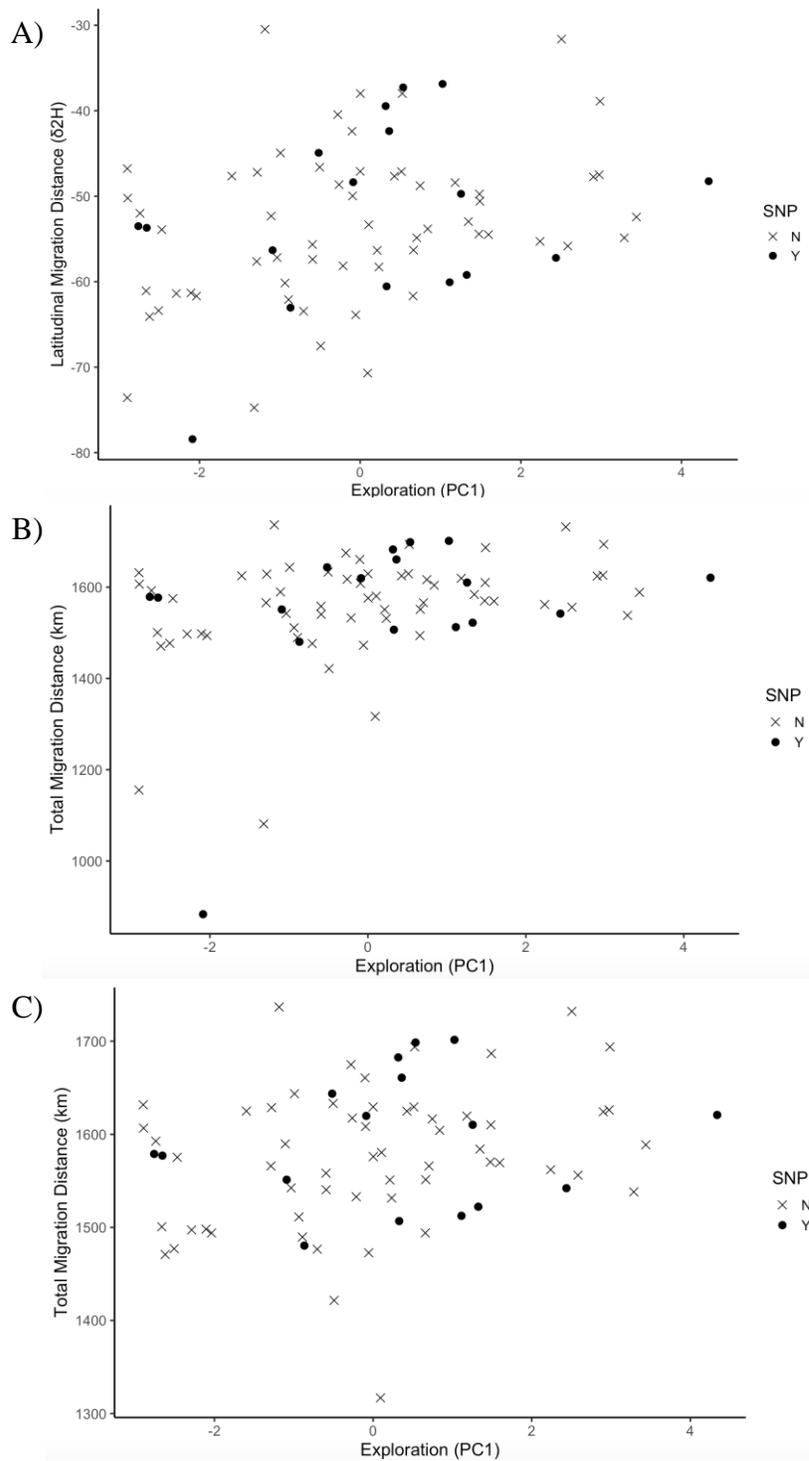
C827T occurrence within exon 3 of the DRD4 gene in relation to exploration score (PC1) and (A) latitudinal migration distance (inferred from $\delta^{2}\text{H}$ values; less negative values indicate more southerly overwinter location and thus longer latitudinal distance) or (B) total migration distance (inferred from likely overwintering origins), (C) total migration distance excluding three northernmost values. Data from 77 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. No difference between individuals with and without the minor allele at C827T was found in exploration scores or latitudinal migration distance, whereas individuals bearing the minor allele at C827T had longer total migration distances when including the three northernmost values. C827T corresponds to C830T in great tits (*Parus major*), a polymorphism that has been associated with differences in novelty seeking behaviours (Fidler et al., 2007; Timm et al., 2019).



the movement behaviours (exploration: Welch's $t_{24.7} = 0.578$, $p = 0.569$; latitudinal migration distance: Welch's $t_{22.9} = 0.387$, $p = 0.703$; total migration distance, full dataset: Welch's $t_{19.4} = -0.172$, $p = 0.865$; total migration distance excluding the three northernmost values: Welch's $t_{25.3} = 0.902$, $p = 0.376$; Figure 3.10).

Figure 3.10. Occurrence of any SNP within exon 3 of the DRD4 gene in relation to exploration score and latitudinal migration distance or total migration distance.

Any SNP occurrence within exon 3 of the DRD4 gene in relation to exploration score (PC1) and (A) latitudinal migration distance (inferred from $\delta^2\text{H}$ values; less negative values indicate more southerly overwinter location and thus longer latitudinal distance); (B) total migration distance (inferred from likely overwintering origins), full dataset; or (C) total migration distance excluding three northernmost values. Data from 77 song sparrows (*Melospiza melodia*) caught near Newboro, Ontario in April-May 2018 and 2019. Individuals bearing a minor allele at any of the SNPs did not differ from those lacking any SNPs in their exploration scores, latitudinal migration distance, or total migration distance.



4. Discussion

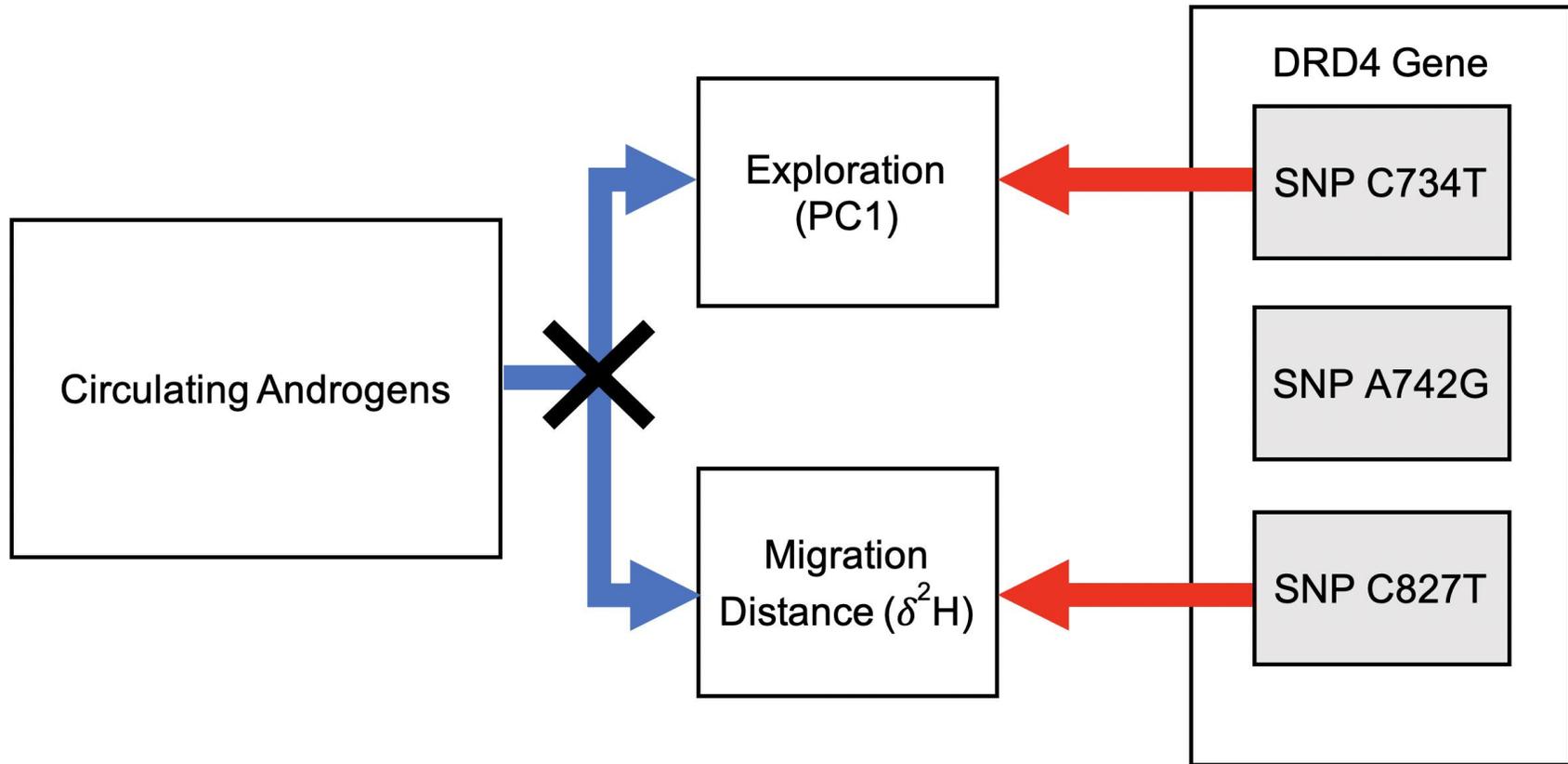
4.1 Overview

Exploratory scores exhibited a significant positive correlation with both latitudinal and total migration distance, supporting the prediction that these movement behaviours on different spatial scales would be related. This relationship was also robust to excluding the three northernmost datapoints, and also to correcting for possible covariates. Unexpectedly, however, the latter analysis showed that the date of first capture was negatively related to both latitudinal and total migration distance, meaning that individuals that were captured earlier in the season had migrated further distances.

Correlations between different behaviours are often assumed to result from a common proximate mechanism underlying the behaviours. I examined two candidate proximate mechanisms, circulating androgen levels and sequence variation at the dopamine receptor DRD4 locus. Contrary to my hypothesis, circulating androgen concentrations were not significantly related to either exploration or to latitudinal or total migration distance the previous winter. Thus, individual variation in androgen levels does not appear to explain the observed relationship between exploratory behaviour and migration distance (Figure 4.1). At the DRD4 exon 3 locus, I identified 6 different SNPs, two of which were associated with differing levels of movement in the population. Song sparrows with SNP C734T explored significantly more on average than those that did not bear this minor allele. A different variant was associated with migration distance: birds bearing the minor allele at C827T, a polymorphism equivalent to SNP C830T which has been associated with novelty seeking behaviours in great tits (Fidler et al., 2007; Timm et al., 2019), had farther total migration distances on average than birds that did not bear the

Figure 4.1 Resulting relationships between proximate mechanisms hypothesized to mediate the relationship between exploratory behaviour and migration distance.

Circulating androgen concentrations did not show a relationship with either exploratory behaviour or migration distance in song sparrows (*Melospiza melodia*). Variation at two SNPs in the DRD4 exon 3, C734T and C827T, was significantly associated with exploratory behaviour and migration distance, respectively. The potential effect of variation in the song sparrow DRD4 gene may be a proximate mechanism linking the two movement behaviours on different geographic scales.



minor allele. These patterns appear to implicate variation at DRD4 in explaining movement at different spatial scales. However, the association between C827T and total migration distance was not robust to excluding the three northernmost datapoints. Moreover, because neither variant was significantly associated with both exploration and migration, it would be premature to conclude that sequence variation at this locus is responsible for the observed relationship between small- and large-scale movements.

4.2 Relationship between exploration and migration distance

Exploratory behaviour scores were positively correlated with both latitudinal and total migration distance, regardless of whether the three northernmost datapoints were excluded. This result supports my prediction that these two movement behaviours would be part of a single behavioural (movement) syndrome. Although I did not test other important movement-related behaviours, it is possible that this movement syndrome might also include natal dispersal, defined as the movement between the birthplace and first breeding place (Dingemanse et al., 2003). Indeed, natal dispersal is positively related to exploratory behaviour in great tits (Dingemanse et al., 2003), and with migration distance in song sparrows (Kelly et al., 2016). Natal dispersal distance in song sparrows is typically on the order of 10 km or less (Zink & Dittmann, 1993), making the spatial scale of this behaviour intermediate between exploration and migration. Thus, the results of my study show that there is a very wide range in the spatial scale over which correlations between movement behaviours arise.

Exploration scores were not significantly repeatable between first and second trials of individuals caught and tested twice in the same year. One possible explanation

for this is that the first test of each individual measured exploration of a truly novel environment, while the second test measured movement with previous experience of the environment. Thus, even though first and second tests were conducted under identical conditions, the birds' different reactions to the novel versus the familiar test environment may have reduced repeatability. Although exploratory behaviour in the novel environment (i.e. first-time test response) was not significantly related to movement behaviour on subsequent tests, the strong relationship between first-time exploration and migration distance suggests that exploration is likely a consistent trait within individuals.

The connection found between exploration and migration was robust to the inclusion of potentially confounding variables (sex, age, year, tarsus length, and date of first capture) that were included in the linear regression models. Regardless of whether these variables were included, exploration showed a significant positive relationship with both latitudinal and total migration distance. Consistent with previous work on this and other species (Martell et al., 2001; Kelly et al., 2016; Lymburner et al., 2016), these multivariate regressions also identified a sex difference in latitudinal migration distance, with males overwintering farther north than females. This pattern is likely due to selection favouring male birds that return to the breeding grounds early enough to obtain high-quality territories, and/or to the larger size of male birds enabling them to withstand colder winter temperatures than females (Brown & Brown, 1998).

The relationship between exploration and migration distance may have been shaped by adaptation, as different combinations of short- and long-range movement strategies may work better together. Exploration allows animals to gain knowledge of their surrounding environment (Renner, 1990; Verbeek et al., 1994). Shorter distance

migrants likely overwinter in habitats that are relatively similar to those of the breeding grounds. Such individuals may also have higher territorial fidelity at the breeding grounds (lower adult dispersal distance) relative to later arriving individuals, as has been reported for prairie warblers (*Dendroica discolor*; Walton & Nolan, 1986). If so, rapid and extensive exploration may be less beneficial for individuals that migrate shorter distances, because the information they gather from exploratory behaviour might carry over between years on both the breeding grounds and overwintering sites. Conversely, longer distance migrants likely encounter a greater variation in habitats the farther they migrate from the breeding grounds, and when returning to the breeding grounds may have to explore more to find territories not occupied by the early-returning individuals that migrated shorter distances.

4.2.1 *Exploration, migration, and date of first capture*

As expected, exploration was related positively to both latitudinal and total migration distance. However, an unexpected and apparently counterintuitive finding was that the date of first capture was negatively related to both measures of migration distance, that is, birds returning from farther south tended to have their first capture date earlier in the spring. Date of first capture is often used as a proxy for arrival date in migratory birds (Garamszegi et al., 2014; Tonra et al., 2011; Lymburner et al., 2016), and all else being equal, shorter-distance migrants are expected to arrive earlier at the breeding grounds than longer-distance migrants.

In this study, I did not initiate capturing song sparrows until mid-April, by which time most or all of the breeding population could have already returned from migration. If so, this would obscure any relationship between date of first capture and arrival date. Indeed, song sparrows are often heard in southern Ontario during early to mid-March. Thus, the date of first capture might not be a reliable measure of arrival date in this study, explaining why no positive relationship was found between migration distance and first capture date. To explain why this relationship was significantly negative, I emphasize the observed correlation between migration and exploration. It is possible that relatively exploratory individuals, shown here to migrate farther distances, may enter the traps more readily and are therefore captured earlier than less exploratory individuals. If true, this pattern illustrates the importance of considering individual variation in behaviour before inferring arrival dates from capture records.

4.3 Relationship between movement and circulating androgens

Circulating androgen concentrations in song sparrows do not appear to represent a shared mechanism underlying both exploration and migration distance. First, androgens did not show the predicted negative relationship with exploratory behaviour. This direction was predicted because aggression, an androgen-related behaviour (Wingfield & Soma, 2002), is negatively associated with exploration in other birds (Drent et al., 1996; Marchetti & Drent, 2000). Conversely, however, a study in great tits suggested that androgens may be positively associated with exploration, because more thorough explorers had higher baseline testosterone levels than superficial explorers (van Oers et al., 2011). Regardless, I detected no relationship between circulating androgen levels and

exploration, despite these data (blood sampling and behavioural testing) usually being collected on the same day. However, I do not discount the possibility that there may be a more complex relationship between androgens and exploration than could be detected in this study. Androgen levels vary seasonally, over the course of the day, and in response to the changing social environment (Wingfield et al., 1990), making them challenging to characterize with confidence.

Neither latitudinal nor total migration distance showed a significant relationship with circulating androgen concentrations. I had predicted that migration distance would show a negative relationship with circulating androgen concentrations upon return to the breeding grounds, as this relationship has been found previously in this population of song sparrows (Lymburner et al., 2016). As noted above, androgen levels can vary seasonally, as well as situationally (Wingfield et al., 1990), making it difficult to confidently assess baseline androgen concentrations. Moreover, if decisions regarding migration distance are made during fall migration, androgen levels several months later (upon returning to the breeding grounds in spring) may no longer reflect fall androgen levels.

Multivariate models addressing the relationship between androgens and small- and large-scale movements also revealed unexpected relationships between each movement behaviour and the date of blood sampling. However, after filtering out individuals whose exploratory behavioural test and blood collection did not occur within five days of each other, date of blood sampling no longer appeared in the model best explaining exploratory behaviour. As suggested above regarding capture date (section 4.2.1), the relationship between date of blood sampling and both movement-related

behaviours may reflect variation in readiness to enter the traps (itself reflecting exploratory tendency). Thus, more exploratory individuals may more readily enter the traps set in their territory relative to less exploratory individuals, and because blood sampling was generally conducted on first capture, exploratory individuals may have been the first to undergo blood collection.

4.4 *Sequence variation at DRD4 exon 3 associated with movement behaviours*

4.4.1 *Comparing DRD4 exon 3 variation between species*

The amount of variation found in exon 3 of the song sparrow DRD4 gene was lower than expected, compared to other avian species, both in the number of SNPs detected and in the frequency of these minor alleles. Yellow-crowned bishops (*Euplestes afer*) sequenced at the same portion of DRD4 that I surveyed (exon 3) show 16 different SNPs (Mueller et al., 2014), in contrast to the 6 SNPs detected in song sparrows, despite fairly similar numbers of individuals genotyped (N = 100 yellow-crowned bishops, N = 77 song sparrows). In collared flycatchers (*Ficedula albicollis*), Garamszegi et al. (2014) reported only two SNPs in the same sequenced region, but both of these were present at higher frequency than any of the SNPs detected in song sparrows (66 of 204 [32.4%] and 49 of 204 [24%] for the two collared flycatcher SNPs, in contrast to minor allele frequencies of 1.3–6.5% for song sparrows).

Comparing the consensus sequence for song sparrow DRD4 exon 3 to that of the great tit ortholog, I identified 12 synonymous and 5 non-synonymous single-nucleotide differences between the species, plus a 3 bp (in-frame) insertion in the great tit sequence relative to that of the song sparrow. Aligning with other published DRD4 sequences for

birds indicated that the insertion is also found in two other members of family *Paridae* (Tibetan ground tit, *Pseudopodoces humilis*; blue tit, *Cyanistes caeruleus*) but not in the white-throated sparrow (*Zonotrichia albicollis*), a member of the *Passerellidae* family to which song sparrows belong. Song sparrows and white-throated sparrows also shared the same consensus amino acid sequence, with both having the same five non-synonymous polymorphisms relative to the great tit sequence. It remains to be seen whether these differences in amino acids alter the function of the resulting receptor proteins, but the similarities between the members of the *Paridae* family and between those of the *Passerellidae* family demonstrate that these differences appear to be evolutionarily conserved. Thus, it is possible that family-level differences in protein sequence and function may underlie family-level differences in exploration and novelty seeking behaviour. Further study is required to test this hypothesis.

4.4.2 *Movement behaviours and DRD4 exon 3 polymorphisms*

When investigating specific SNPs, song sparrows bearing the minor allele at C734T had significantly higher exploratory scores on average than those without the SNP at this site. It is important to note that though a small sample size (4 individuals bearing the minor allele) could have generated a spurious relationship, it is at least possible that this variant may influence exploration. Previous studies have found relationships between polymorphisms in this exon related to exploration and other novelty seeking behaviours in other bird species (Fidler et al., 2007; Garamszegi et al., 2014; Timm et al., 2019). The involvement of this dopamine receptor in the reward and motivational pathway suggests that sequence variation may influence exploratory movements and novelty seeking.

The effects of sequence variation on avian D4 dopamine receptors are not well known, especially for synonymous SNPs such as C734T that do not alter amino acid sequence. Though they do not change protein sequence, synonymous variants in DNA sequence can affect mRNA translation, with implications for the stability of protein structure, and even gene expression (Chamary et al., 2006). The effects of synonymous polymorphisms have been documented in the human DRD2 gene, where one such SNP decreased mRNA translation and stability, and reduced the response of dopamine-induced up-regulation of the DRD2 receptor (Duan et al., 2003).

Another synonymous polymorphism that I assessed, C827T, was significantly associated with total migration distance, at least when the full dataset was used. Individuals bearing the minor allele had significantly longer total migration distances than those that did not. This is the first evidence of a relationship between migration distance and a polymorphism in the DRD4 gene. This polymorphism is especially interesting because it corresponds to the C830T polymorphism in great tits that has been implicated in novelty seeking behaviour (Timm et al., 2019) and exploratory behaviour (Fidler et al., 2007). The results of my study demonstrate that this SNP may also influence other movement behaviours such as migration, and that this gene and the motivational and reward pathway may be tied to movement behaviours on multiple geographic scales.

In contrast to the associations noted above, variation at SNPs C734T or A742G was not associated with total migration distance. Similarly, none of the three SNPs examined (C734T, A742G, or C827T) predicted variation in latitudinal migration distance. Finally, exploratory behaviour was not associated with genotype at SNP A742G

or C827T. I had expected a priori that C827T might be linked to exploration, because as noted above, this SNP corresponds to C830T in great tits, a polymorphism linked to exploration and novelty seeking in that species (Fidler et al., 2007; Timm et al., 2019). Failing to find significant differences in exploration between song sparrows with and without this SNP may reflect low statistical power due to small sample size (only 4 individuals bearing the minor allele at C827T). Alternatively, however, C827T may not affect exploratory behaviour in song sparrows. Riyahi et al. (2017) found no relationship between exploration and the C830T polymorphism in a Spanish population of great tits. Moreover, this SNP does not occur in all species. For example, it was not one of the 16 SNPs identified in the collard flycatcher by Garamszegi et al. (2014).

Overall, the generally low incidence of variation at DRD4 in this population of song sparrows, and the absence of any variants associated with both exploration and migration distance, does not provide overwhelming confidence that DRD4 represents a shared proximate mechanism influencing both exploration and migration in song sparrows and mediating the relationship between them. However, despite the low sample size, I observed SNP-related variation in each of the movement behaviours examined. Higher exploratory scores and longer total migration distances of those individuals bearing the minor allele at C734T and C827T, respectively, suggests that the DRD4 locus should be investigated further for its role in linking these two movement behaviours, and potentially movements over different spatial scales, such as dispersal.

4.5 *Future studies*

Both exploratory behaviour (this study) and natal dispersal (Kelly et al., 2016) have been shown to be positively related to seasonal migration distance in song sparrows. In order to further test the hypothesis that these three behaviours are part of the same movement syndrome, future research should investigate whether natal dispersal and exploration are correlated as well in this species. There is evidence for a positive relationship between exploration and dispersal in great tits (Dingemanse et al., 2003), providing a basis for this predicted relationship in song sparrows.

In addition to finding a relationship between exploration and migration distance, I found that both exploratory scores and total migration distance were associated with variation in the DRD4 gene. Though novelty seeking behaviours, including exploration, have been linked to polymorphisms in the DRD4 gene in previous studies (Fidler et al., 2007; Timm et al., 2019), this is the first study to link sequence variation at DRD4 to variation in migration distance. The low levels of DRD4 polymorphisms observed in this study suggest that further research is needed on the effect of variation in this gene on exploration and migration, and may also suggest that song sparrows have been less subject to balancing selection at DRD4 relative to other species that have been investigated. A valuable next step would be to conduct a comparative study of the DRD4 gene in relation to movement behaviours of multiple migratory birds, to investigate if this relationship exists in other species in addition to the song sparrow. Examining patterns of sequence variation at DRD4 across migratory and non-migratory species could also shed light on patterns of molecular selection at this locus. In addition, genotyping and investigating the association between genes previously linked to migration, such as

Adcyap1 (Bazzi et al., 2016), and exploratory behaviour would further add to our understanding of the genetics of movement behaviours

Finally, I detected no relationship between circulating androgens and movement behaviour in the present study, in contrast to findings of Lymburner et al. (2016) in this same population. Factors such as handling and time of day could have affected the androgen concentrations measured, obscuring any underlying relationship between baseline androgen levels and movement. To mitigate some of these sources of variation, future studies could measure more repeatable levels of androgen, instead of relying on baseline levels which are subject to fluctuation, and determine whether these are correlated with movement behaviours. For example, gonadotropin-releasing hormone (GnRH) challenges produce a short-term increase in testosterone, similar to levels shown in territorial interactions (McGlothlin et al., 2007); the maximal testosterone level is presumably less subject to fluctuation than baseline level. Such tests may provide a more consistent metric to assess the relationship between testosterone or other androgens and movement behaviours. Alternatively, androgens could be administered endogenously then exploratory behaviour and/or migration-related behaviour (e.g. nocturnal restlessness, departure date, migration distance or speed) could be monitored to assess these relationships.

4.6 Conclusions

While relationships have been reported in birds between movement behaviours such as exploration and dispersal (Dingemanse et al., 2003), and dispersal and migration distance (Kelly et al., 2016), my research is the first to demonstrate a relationship

between exploratory behaviour and migration distance. Song sparrows that migrated longer distances also had higher exploratory scores. The relationship between these two behaviours, occurring on very different spatial scales, supports the idea that these movement behaviours form part of the same movement syndrome, one that likely includes natal dispersal as well.

Of the two proximate mechanisms hypothesized to mediate this relationship, variation in the DRD4 gene exon 3 is the most likely to affect exploration and migration. Androgen concentrations did not show a relationship with either movement behaviour, whereas one polymorphism at DRD4 exon 3 was associated with variation in exploratory behaviour and a second polymorphism associated with variation in total migration distance. Although previous studies have demonstrated relationships between androgens and migration distance (Lymburner et al., 2016) and between androgens and exploration (van Oers et al., 2011), my results suggest that androgenic hormones have less of an effect on movement behaviours than predicted and cannot explain the observed relationship between exploration and migration.

The relationship between novelty seeking behaviours and variation at the DRD4 gene has been well documented in avian species (Fidler et al., 2007; Garamszegi et al., 2014; Mueller et al., 2014; Riyahi et al., 2017; Timm et al., 2019). However, my study is the first to link variation in DRD4 sequence to variation in migration distance, while also linking a second variant to variation in exploratory tendency. Overall, my findings demonstrate a novel relationship between exploration and migration distance, and suggest that variation in the DRD4 gene may represent a shared proximate mechanism resulting in the coupling of these behaviours occurring over strikingly different spatial scales.

5. References

- Arcese, P., Sogge, M. K., Marr, A. B., & Patten, M. A. (2002). Song Sparrow (*Melospiza melodia*). *The Birds of North America Online*. <https://doi.org/10.2173/bna.704>
- Bartoń, K. (2019). *MuMIn: Multi-Model Inference*. R package version 1.43.10. <https://cran.r-project.org/package=MuMIn>
- Bazzi, G., Galimberti, A., Hays, Q. R., Bruni, I., Cecere, J. G., Gianfranceschi, L., Hobson, K. A., Morbey, Y. E., Saino, N., Guglielmo, C. G., & Rubolini, D. (2016). Adcyap1 polymorphism covaries with breeding latitude in a Nearctic migratory songbird, the Wilson's warbler (*Cardellina pusilla*). *Ecology and Evolution*, 6(10), 3226–3239. <https://doi.org/10.1002/ece3.2053>
- Bell, A. M. (2005). Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology*, 18(2), 464–473. <https://doi.org/10.1111/j.1420-9101.2004.00817.x>
- Bell, A. M. (2007). Future directions in behavioural syndromes research. *Proceedings of the Royal Society B: Biological Sciences*, 274(1611), 755–761. <https://doi.org/10.1098/rspb.2006.0199>
- Berthold, P., Wiltschko, W., Miltenberger, H., & Querner, U. (1990). Genetic transmission of migratory behavior into a nonmigratory bird population. *Experientia*, 46(1), 107–108. <https://doi.org/10.1007/BF01955432>
- Berthold, Peter. (1988). Evolutionary aspects of migratory behavior in European warblers. *Journal of Evolutionary Biology*, 1(3), 195–209. <https://doi.org/10.1046/j.1420-9101.1998.1030195.x>
- Berthold, Peter, Pulido, F., Berthold, P., & Pulido, F. (1994). Heritability of migratory

activity in a natural bird population. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 257(1350), 311–315.

<https://doi.org/10.1098/rspb.1994.0131>

Biondi, L. M., Bó, M. S., & Vassallo, A. I. (2010). Inter-individual and age differences in exploration, neophobia and problem-solving ability in a Neotropical raptor (*Milvago chimango*). *Animal Cognition*, 13(5), 701–710. <https://doi.org/10.1007/s10071-010-0319-8>

BirdLife International and Handbook of the Birds of the World (2019) Bird species distribution maps of the world. Version 2019.1. Available at <http://datazone.birdlife.org/species/requestdis>. (2019).

Bowen, G. J., Wassenaar, L. I., & Hobson, K. A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, 143(3), 337–348. <https://doi.org/10.1007/s00442-004-1813-y>

Brown, C. R., & Brown, M. B. (1998). Intense natural selection on body size and wing and tail asymmetry in Cliff Swallows during severe weather. *Evolution*, 52(5), 1461. <https://doi.org/10.2307/2411315>

Carbó-Ramírez, P., & Zuria, I. (2015). Immune condition and blood parasites in three sparrow species with different migratory status in central Mexico. *Avian Biology Research*, 8(3), 167–174. <https://doi.org/10.3184/175815515X14371521830098>

Chamary, J. V., Parmley, J. L., & Hurst, L. D. (2006). Hearing silence: Non-neutral evolution at synonymous sites in mammals. *Nature Reviews Genetics*, 7(2), 98–108. <https://doi.org/10.1038/nrg1770>

Cloninger, C. R., Adolfsson, R., & Svrakic, N. M. (1996). Mapping genes for human

- personality. *Nature Genetics*, 12(1), 3–4. <https://doi.org/10.1038/ng0196-3>
- Coplen, T. B. (2017a). Report of stable isotopic composition reference material CBS (Caribou Hoof Standard) (Hydrogen and oxygen isotopes in caribou hoof keratin). *United States Geological Survey*, 1–3.
- Coplen, T. B. (2017b). Report of stable isotopic composition reference material KHS (Kudu Horn Standard) (Hydrogen and oxygen isotopes in kudu horn keratin). *United States Geological Survey*, 1–3.
<http://dx.doi.org/10.1002/rcm.5129>
<http://doi.wiley.com/10.1002/rcm.5129>
- Davis, A., & Arcese, P. (1999). An examination of migration in Song Sparrows using banding recovery data. *North American Bird Bander*, 24(4), 122–128.
- Dingemanse, N. J., Both, C., Drent, P. J., Van Oers, K., & Van Noordwijk, A. J. (2002). Repeatability and heritability of exploratory behaviour in Great Tits from the wild. *Animal Behaviour*, 64(6), 929–938. <https://doi.org/10.1006/anbe.2002.2006>
- Dingemanse, N. J., Both, C., Van Noordwijk, A. J., Rutten, A. L., & Drent, P. J. (2003). Natal dispersal and personalities in Great Tits (*Parus major*). *Proceedings of the Royal Society B: Biological Sciences*, 270(1516), 741–747.
<https://doi.org/10.1098/rspb.2002.2300>
- Dingemanse, N. J., & De Goede, P. (2004). The relation between dominance and exploratory behavior is context-dependent in wild Great Tits. *Behavioral Ecology*, 15(6), 1023–1030. <https://doi.org/10.1093/beheco/arh115>
- Dingemanse, N. J., Dochtermann, N. A., & Nakagawa, S. (2012). Defining behavioural syndromes and the role of “syndrome deviation” in understanding their evolution. *Behavioral Ecology and Sociobiology*, 66(11), 1543–1548.

<https://doi.org/10.1007/s00265-012-1416-2>

- Drent, P. J., Van Oers, K., & Van Noordwijk, A. J. (2003). Realized heritability of personalities in the Great Tit (*Parus major*). *Proceedings of the Royal Society B: Biological Sciences*, 270(1510), 45–51. <https://doi.org/10.1098/rspb.2002.2168>
- Drent, P. J., Verbeek, M. E. M., & Boon, A. (1996). Exploration, aggressive behaviour and dominance in pair-wise confrontations of juvenile male Great Tits. *Behaviour*, 133, 945–963. <https://doi.org/10.1163/156853996X00314>
- Duan, J., Wainwright, M. S., Comeron, J. M., Saitou, N., Sanders, A. R., Gelernter, J., & Gejman, P. V. (2003). Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Human Molecular Genetics*, 12(3), 205–216. <https://doi.org/10.1093/hmg/ddg055>
- Dufty, A. M. (1989). Testosterone and survival: A cost of aggressiveness? *Hormones and Behavior*, 23(2), 185–193. [https://doi.org/10.1016/0018-506X\(89\)90059-7](https://doi.org/10.1016/0018-506X(89)90059-7)
- Evans, J., Boudreau, K., & Hyman, J. (2010). Behavioural syndromes in urban and rural populations of Song Sparrows. *Ethology*, 116(7), 588–595. <https://doi.org/10.1111/j.1439-0310.2010.01771.x>
- Fidler, A. E., Van Oers, K., Drent, P. J., Kuhn, S., Mueller, J. C., & Kempenaers, B. (2007). DRD4 gene polymorphisms are associated with personality variation in a passerine bird. *Proceedings of the Royal Society B: Biological Sciences*, 274(1619), 1685–1691. <https://doi.org/10.1098/rspb.2007.0337>
- Folstad, I., & Skarstein, F. (1997). Is male germ line control creating avenues for female choice? *Behavioral Ecology*, 8(1), 109–112. <https://doi.org/10.1093/beheco/8.1.109>
- Garamszegi, L. Z., Eens, M., Hurtrez-Boussès, S., & Møller, A. P. (2005). Testosterone,

- testes size, and mating success in birds: A comparative study. *Hormones and Behavior*, 47(4), 389–409. <https://doi.org/10.1016/j.yhbeh.2004.11.008>
- Garamszegi, L. Z., Hirschenhauser, K., Bókony, V., Eens, M., Hurtrez-Boussès, S., Møller, A. P., Oliveira, R. F., & Wingfield, J. C. (2008). Latitudinal distribution, migration, and testosterone levels in birds. *American Naturalist*, 172(4), 533–546. <https://doi.org/10.1086/590955>
- Garamszegi, L. Z., Mueller, J. C., Markó, G., Szász, E., Zsebők, S., Herczeg, G., Eens, M., & Török, J. (2014). The relationship between DRD4 polymorphisms and phenotypic correlations of behaviors in the Collared Flycatcher. *Ecology and Evolution*, 4(8), 1466–1479. <https://doi.org/10.1002/ece3.1041>
- Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. G. (1998). A DNA test to sex most birds. *Molecular Ecology*, 7(8), 1071–1075. <https://doi.org/10.1046/j.1365-294x.1998.00389.x>
- Hegner, R. E., & Wingfield, J. C. (1987). Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male House Sparrows. *The Auk*, 104(3), 462–469. <https://doi.org/10.2307/4087545>
- Helms, C. M., Gubner, N. R., Wilhelm, C. J., Mitchell, S. H., & Grandy, D. K. (2008). D4 receptor deficiency in mice has limited effects on impulsivity and novelty seeking. *Pharmacology Biochemistry and Behavior*, 90(3), 387–393. <https://doi.org/10.1016/j.pbb.2008.03.013>
- Hertel, A. G., Leclerc, M., Warren, D., Pelletier, F., Zedrosser, A., & Mueller, T. (2019). Don't poke the bear: Using tracking data to quantify behavioural syndromes in elusive wildlife. *Animal Behaviour*, 147, 91–104.

<https://doi.org/10.1016/j.anbehav.2018.11.008>

Hijmans, R. J. (2019). *geosphere: Spherical Trigonometry. R package version 1.5-10.*

<https://cran.r-project.org/package=geosphere>

Hijmans, R. J. (2019). *raster: Geographic Data Analysis and Modeling. R package*

version 3.0-7. <https://cran.r-project.org/package=raster>

Hobson, K. A., Van Wilgenburg, S. L., Faaborg, J., Toms, J. D., Rengifo, C., Sosa, A. L.,

Aubry, Y., & Brito Aguilar, R. (2014). Connecting breeding and wintering grounds

of Neotropical migrant songbirds using stable hydrogen isotopes: A call for an

isotopic atlas of migratory connectivity. *Journal of Field Ornithology*, 85(3), 237–

257. <https://doi.org/10.1111/jfo.12065>

Hobson, K. A., Van Wilgenburg, S. L., Wassenaar, L. I., & Larson, K. (2012). Linking

hydrogen ($\delta^2\text{H}$) isotopes in feathers and precipitation: Sources of variance and

consequences for assignment to isoscapes. *PLoS ONE*, 7(4), 1–9.

<https://doi.org/10.1371/journal.pone.0035137>

Hobson, K., & Wassenaar, L. (2019). *Tracking Animal Migration with Stable Isotopes*

(K. Hobson & L. Wassenaar (Eds.); 2nd Editio). Elsevier.

<https://doi.org/10.1016/C2017-0-01125-4>

Huang, P., Kerman, K., Sieving, K. E., & St. Mary, C. M. (2016). Evaluating the novel-

environment test for measurement of exploration by bird species. *Journal of*

Ethology, 34(1), 45–51. <https://doi.org/10.1007/s10164-015-0444-6>

Jenkins, K. D., & Cristol, D. A. (2002). Evidence of differential migration by sex in

White-Throated Sparrows (*Zonotrichia albicollis*). *The Auk*, 119(2), 539–543.

<https://doi.org/10.2307/4089903>

- Karlsson Green, K., Eroukhmanoff, F., Harris, S., Pettersson, L. B., & Svensson, E. I. (2016). Rapid changes in genetic architecture of behavioural syndromes following colonization of a novel environment. *Journal of Evolutionary Biology*, *29*(1), 144–152. <https://doi.org/10.1111/jeb.12769>
- Kassambara, A. (2019). *ggpubr: “ggplot2” Based Publication Ready Plots. R package version 0.2.4.*
- Kelly, T. R., Hobson, K. A., Casbourn, G. W., MacDougall-Shackleton, E. A., & MacDougall-Shackleton, S. A. (2019). Long-term winter-site fidelity in Song Sparrows (*Melospiza melodia*). *Auk*, *136*(2), 1–12. <https://doi.org/10.1093/auk/ukz010>
- Kelly, T. R., MacGillivray, H. L., Sarquis-Adamson, Y., Watson, M. J., Hobson, K. A., & MacDougall-Shackleton, E. A. (2016). Seasonal migration distance varies with natal dispersal and predicts parasitic infection in Song Sparrows. *Behavioral Ecology and Sociobiology*, *70*(11), 1857–1866. <https://doi.org/10.1007/s00265-016-2191-2>
- Ketterson, E. D., & Nolan, V. (1992). Hormones and life histories: an integrative approach. *American Naturalist*, *140*(Suppl.), 33–62. <https://doi.org/10.1086/285396>
- Lessells, C. M., & Boag, P. T. (1987). Unrepeatable repeatabilities: A common mistake. *The Auk*, *104*(1), 116–121. <https://doi.org/10.2307/4087240>
- Lymburner, A. H., Kelly, T. R., Hobson, K. A., MacDougall-Shackleton, E. A., & MacDougall-Shackleton, S. A. (2016). Testosterone, migration distance, and migratory timing in Song Sparrows *Melospiza melodia*. *Hormones and Behavior*, *85*, 102–107. <https://doi.org/10.1016/j.yhbeh.2016.06.013>

- Marchetti, C., & Drent, P. J. (2000). Individual differences in the use of social information in foraging by captive Great Tits. *Animal Behaviour*, *60*(1), 131–140. <https://doi.org/10.1006/anbe.2000.1443>
- Marchetti, C., & Zehtindjiev, P. (2009). Migratory orientation of Sedge Warblers (*Acrocephalus schoenobaenus*) in relation to eating and exploratory behaviour. *Behavioural Processes*, *82*(3), 293–300. <https://doi.org/10.1016/j.beproc.2009.07.008>
- Martell, M. S., Henny, C. J., Nye, P. E., & Solensky, M. J. (2001). Fall migration routes, timing, and wintering sites of North American ospreys as determined by satellite telemetry. *The Condor*, *103*(4), 715. [https://doi.org/10.1650/0010-5422\(2001\)103\[0715:fmrtaw\]2.0.co;2](https://doi.org/10.1650/0010-5422(2001)103[0715:fmrtaw]2.0.co;2)
- Martins, E. P., & Bhat, A. (2014). Population-level personalities in zebrafish: Aggression-boldness across but not within populations. *Behavioral Ecology*, *25*(2), 368–373. <https://doi.org/10.1093/beheco/aru007>
- Mazerolle, D. F., & Hobson, K. A. (2005). Estimating origins of short-distance migrant songbirds in North America: Contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. *The Condor*, *107*(2), 280–288. <https://doi.org/10.1650/7681>
- Mazerolle, M. J. (2006). Improving data analysis in herpetology: Using Akaike's information criterion (AIC) to assess the strength of biological hypotheses. *Amphibia Reptilia*, *27*(2), 169–180. <https://doi.org/10.1163/156853806777239922>
- McGlothlin, J. W., Jawor, J. M., & Ketterson, E. D. (2007). Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. *American*

- Naturalist*, 170(6), 864–875. <https://doi.org/10.1086/522838>
- Moore, M. (1984). Changes in territorial defense produced by changes in circulating levels of testosterone: A possible hormonal basis for mate-guarding behavior in White-Crowned Sparrows. *Behavior*, 88, 215–226.
- Moretz, J. A., Martins, E. P., & Robison, B. D. (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral Ecology*, 18(3), 556–562. <https://doi.org/10.1093/beheco/arm011>
- Mueller, J. C., Edelaar, P., Carrete, M., Serrano, D., Potti, J., Blas, J., Dingemanse, N. J., Kempenaers, B., & Tella, J. L. (2014). Behaviour-related DRD4 polymorphisms in invasive bird populations. *Molecular Ecology*, 23(11), 2876–2885. <https://doi.org/10.1111/mec.12763>
- Mukhin, A., Kosarev, V., & Ktitorov, P. (2005). Nocturnal life of young songbirds well before migration. *Proceedings of the Royal Society B: Biological Sciences*, 272(1572), 1535–1539. <https://doi.org/10.1098/rspb.2005.3120>
- Myers, R. E., & Hyman, J. (2016). Differences in measures of boldness even when underlying behavioral syndromes are present in two populations of the Song Sparrow (*Melospiza melodia*). *Journal of Ethology*, 34(3), 197–206. <https://doi.org/10.1007/s10164-016-0465-9>
- Netter, P. (2006). Dopamine challenge tests as an indicator of psychological traits. *Human Psychopharmacology*, 21(2), 91–99. <https://doi.org/10.1002/hup.754>
- Ogurtsov, S. V., Antipov, V. A., & Permyakov, M. G. (2018). Sex differences in exploratory behaviour of the Common Toad, *Bufo bufo*. *Ethology Ecology and Evolution*, 30(6), 543–568. <https://doi.org/10.1080/03949370.2018.1459864>

- Pulido, F. (2007). The genetics and evolution of avian migration. *BioScience*, *57*(2), 165–174. <https://doi.org/10.1641/b570211>
- Renner, M. J. (1990). Neglected aspects of exploratory and investigatory behavior. *Psychobiology*, *18*(1), 16–22. <https://doi.org/10.3758/BF03327209>
- Riyahi, S., Björklund, M., Mateos-Gonzalez, F., & Senar, J. C. (2017). Personality and urbanization: behavioural traits and DRD4 SNP830 polymorphisms in Great Tits in Barcelona city. *Journal of Ethology*, *35*(1), 101–108. <https://doi.org/10.1007/s10164-016-0496-2>
- RStudio Team. (2019). *RStudio: Integrated Development for R*. RStudio, Inc., Boston, MA. Version 1.2.1578. <http://www.rstudio.com/>
- Schmaljohann, H., Becker, P. J. J., Karaardic, H., Liechti, F., Naef-Daenzer, B., & Grande, C. (2011). Nocturnal exploratory flights, departure time, and direction in a migratory songbird. *Journal of Ornithology*, *152*(2), 439–452. <https://doi.org/10.1007/s10336-010-0604-y>
- Schmidt, K. L., MacDougall-Shackleton, E. A., Soma, K. K., & MacDougall-Shackleton, S. A. (2014). Developmental programming of the HPA and HPG axes by early-life stress in male and female Song Sparrows. *General and Comparative Endocrinology*, *196*, 72–80. <https://doi.org/10.1016/j.ygcen.2013.11.014>
- Schuett, W., & Dall, S. R. X. (2009). Sex differences, social context and personality in Zebra Finches, *Taeniopygia guttata*. *Animal Behaviour*, *77*(5), 1041–1050. <https://doi.org/10.1016/j.anbehav.2008.12.024>
- Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: An ecological and evolutionary overview. *Trends in Ecology and Evolution*, *19*(7), 372–378.

<https://doi.org/10.1016/j.tree.2004.04.009>

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: An integrative overview. *Quarterly Review of Biology*, *79*(3), 241–277.

<https://doi.org/10.1086/422893>

Sih, A., & Del Giudice, M. (2012). Linking behavioural syndromes and cognition: A behavioural ecology perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1603), 2762–2772. <https://doi.org/10.1098/rstb.2012.0216>

Sih, A., Kats, L. B., & Maurer, E. F. (2003). Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. *Animal Behaviour*, *65*(1), 29–44. <https://doi.org/10.1006/anbe.2002.2025>

Stecher, G., Tamura, K., & Kumar, S. (2020). Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution*, *37*(4), 1237–1239.

<https://doi.org/10.1093/molbev/msz312>

Tibblin, P., Forsman, A., Borger, T., & Larsson, P. (2016). Causes and consequences of repeatability, flexibility and individual fine-tuning of migratory timing in pike.

Journal of Animal Ecology, *85*(1), 136–145. <https://doi.org/10.1111/1365-2656.12439>

Timm, K., Mägi, M., Telve, K., & Tilgar, V. (2019). The behavioural response of Great Tits to novel environment and handling is affected by the DRD4 gene. *Ibis*, *161*(1),

91–100. <https://doi.org/10.1111/ibi.12604>

Tonra, C. M., Marra, P. P., & Holberton, R. L. (2011). Migration phenology and winter habitat quality are related to circulating androgen in a long-distance migratory bird.

Journal of Avian Biology, *42*(5), 397–404. <https://doi.org/10.1111/j.1600->

048X.2011.05333.x

- Torchiano, M. (2019). *effsize: Efficient Effect Size Computation*. doi: 10.5281/zenodo.1480624 (URL: <https://doi.org/10.5281/zenodo.1480624>), R package version 0.7.6. <https://doi.org/10.5281/zenodo.1480624>
- van Oers, K., Buchanan, K. L., Thomas, T. E., & Drent, P. J. (2011). Correlated response to selection of testosterone levels and immunocompetence in lines selected for avian personality. *Animal Behaviour*, 81(5), 1055–1061. <https://doi.org/10.1016/j.anbehav.2011.02.014>
- Van Wilgenburg, S. L., & Hobson, K. A. (2011). Combining stable-isotope (δD) and band recovery data to improve probabilistic assignment of migratory birds to origin. *Ecological Applications*, 21(4), 1340–1351. <https://doi.org/10.1890/09-2047.1>
- Verbeek, M. E. M., Drent, P. J., & Wiepkema, P. R. (1994). Consistent individual differences in early exploratory behaviour of male Great Tits. *Animal Behaviour*, 48(5), 1113–1121. <https://doi.org/10.1006/anbe.1994.1344>
- Verhulst, E. C., Mateman, A. C., Zwier, M. V., Caro, S. P., Verhoeven, K. J. F., & van Oers, K. (2016). Evidence from pyrosequencing indicates that natural variation in animal personality is associated with DRD4 DNA methylation. *Molecular Ecology*, 25(8), 1801–1811. <https://doi.org/10.1111/mec.13519>
- Walton, R., & Nolan, V. (1986). Imperfect information and the persistence of pretenders: Male Prairie Warblers contesting for territory. *The American Naturalist*, 128(3), 427–432. <https://doi.org/10.1086/284574>
- Warnes, G., Gorjanc, G., Leisch, F., & Man, M. (2019). *genetics: Population Genetics*. R package version 1.3.8.1.2. <https://cran.r-project.org/package=genetics>

- Wassenaar, L. I., & Hobson, K. A. (2000). Stable-carbon and hydrogen isotope ratios reveal breeding origins of Red-Winged Blackbirds. *Ecological Applications*, *10*(3), 911. <https://doi.org/10.2307/2641055>
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag New York, ISBN 978-3-319-24277-4
- Wickham, H., François, R., Henry, L., & Müller, K. (2019). *dplyr: A Grammar of Data Manipulation. R package version 0.8.3*. <https://cran.r-project.org/package=dplyr>
- Wingfield, J. C., Hegner, R. E., Dufty, A. M., & Ball, G. F. (1990). The “Challenge Hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *The American Naturalist*, *136*(6), 829–846. <https://doi.org/10.1086/285134>
- Wingfield, J. C., & Soma, K. K. (2002). Spring and autumn territoriality in Song Sparrows: Same behavior, different mechanisms? *Integrative and Comparative Biology*, *42*(1), 11–20. <https://doi.org/10.1093/icb/42.1.11>
- Zink, R. M., & Dittmann, D. L. (1993). Gene flow, refugia, and evolution of geographic variation in the Song Sparrow (*Melospiza melodia*). *Evolution*, *47*(3), 717. <https://doi.org/10.2307/2410178>

6. Appendix A

Table A.1 AICc-ranked selection table for models predicting latitudinal migration distance (inferred from $\delta^2\text{H}$) as a function of exploratory scores and other potential covariates.

Model	<i>df</i>	AICc	ΔAICc
$\delta^2\text{H} \sim \text{Expl PC1} + \text{Date of First Cap} + \text{Sex} + \text{Year}$	6	557.624	0.000
$\delta^2\text{H} \sim \text{Expl PC1} + \text{Date of First Cap} + \text{Sex}$	5	557.708	0.085
$\delta^2\text{H} \sim \text{Expl PC1} + \text{Date of First Cap} + \text{Sex} + \text{Age}$	6	559.457	1.834
$\delta^2\text{H} \sim 1$ (Null Model)	2	573.181	15.557

Note: The fully saturated model calculated latitudinal migration distance as a function of exploration score (PC1), date of first capture, sex, age class, year, and tarsus length. All combinations of these predictors were assessed, but only models within 2 AICc units of the highest-ranked model (i.e. that with the lowest AICc value) are reported in this table, along with the null model. The most parsimonious model was identified as the simplest model within 2 AICc units of the highest-ranked (lowest AICc) model (following Mazerolle, 2006) and is identified here in bold type.

Table A.2 AICc-ranked selection table for models predicting total migration distance using the full dataset as a function of exploratory scores and other potential covariates.

Model	<i>df</i>	AICc	Δ AICc
Distance ~ Expl PC1 + Date of First Cap	4	970.552	0.000
Distance ~ Expl PC1 + Date of First Cap + Year	5	971.526	0.974
Distance ~ Expl PC1 + Date of First Cap + Sex	5	972.253	1.701
Distance ~ Expl PC1 + Date of First Cap + Sex + Tarsus	5	972.429	1.878
Distance ~ 1 (Null Model)	2	983.437	12.885

Note: The fully saturated model calculated total migration distance as a function of exploration score (PC1), date of first capture, sex, age class, year, and tarsus length. All combinations of these predictors were assessed, but only models within 2 AICc units of the highest-ranked model (i.e. that with the lowest AICc value) are reported in this table, along with the null model. The most parsimonious model was identified as the simplest model within 2 AICc units of the highest-ranked (lowest AICc) model (following Mazerolle, 2006) and is identified here in bold type.

Table A.3 AICc-ranked selection table for models predicting total migration distance excluding the three northernmost values as a function of exploratory scores and other potential covariates.

Model	<i>df</i>	AICc	Δ AICc
Distance ~ Expl PC1 + Date of First Cap + Sex	5	856.156	0.000
Distance ~ Expl PC1 + Date of First Cap + Sex + Age	6	856.129	0.027
Distance ~ Expl PC1 + Date of First Cap + Age	5	856.354	0.198
Distance ~ Date of First Cap + Sex + Age	5	856.431	0.275
Distance ~ Expl PC1 + Date of First Cap + Sex + Year	6	856.605	0.449
Distance ~ Date of First Cap + Sex	4	856.812	0.656
Distance ~ Date of First Cap + Age	4	857.036	0.880
Distance ~ Date of First Cap + Sex + Year	5	857.256	1.100
Distance ~ Expl PC1 + Sex + Year	5	857.448	1.292
Distance ~ Expl PC1 + Date of First Cap + Sex + Age + Year	7	857.563	1.407
Distance ~ Date of First Cap + Sex + Age + Year	6	857.842	1.686
Distance ~ Expl PC1 + Age	4	857.877	1.721
Distance ~ Expl PC1 + Sex	4	858.200	1.964
Distance ~ 1 (Null Model)	2	863.031	6.875

Note: The fully saturated model calculated total migration distance as a function of exploration score (PC1), date of first capture, sex, age class, year, and tarsus length. All combinations of these predictors were assessed, but only models within 2 AICc units of the highest-ranked model (i.e. that with the lowest AICc value) are reported in this table, along with the null model. The most parsimonious model was identified as the simplest model within 2 AICc units of the highest-ranked (lowest AICc) model (following Mazerolle, 2006) and is identified here in bold type.

Table A.4 AICc-ranked selection table for models predicting exploration scores as a function of logarithmically transformed androgen concentration and other potential covariates.

Model	<i>df</i>	AICc	Δ AICc
Expl ~ Date of Bleed	3	305.405	0.000
Expl ~ Date of Bleed + Sex	4	305.908	0.503
Expl ~ Date of Bleed + log[Andr]	4	306.396	0.992
Expl ~ Date of Bleed + Tarsus	4	307.317	1.912
Expl ~ 1 (Null Model)	2	308.342	2.938

Note: The fully saturated model calculated exploration scores as a function of logarithmically transformed androgen concentrations, date of bleed, sex, year, and tarsus length. All combinations of these predictors were assessed, but only models within 2 AICc units of the highest-ranked model (i.e. that with the lowest AICc value) are reported in this table, along with the null model. The most parsimonious model was identified as the simplest model within 2 AICc units of the highest-ranked (lowest AICc) model (following Mazerolle, 2006) and is identified here in bold type.

Table A.5 AICc-ranked selection table for models predicting exploration scores as a function of logarithmically transformed androgen concentration and other potential covariates, for the subset of individuals that had blood collected and behavioural testing done within five days of one another.

Model	<i>df</i>	AICc	Δ AICc
Expl ~ Date of Bleed	3	267.552	0.000
Expl ~ Date of Bleed + Sex	4	267.754	0.020
Expl ~ Date of Bleed + log[Andr]	4	268.001	0.449
Expl ~ 1 (Null Model)	2	268.413	0.861
Expl ~ log[Andr]	3	269.247	1.695
Expl ~ Date of Bleed + log[Andr] + Sex	5	269.321	1.769
Expl ~ Sex	3	269.328	1.776
Expl ~ Date of Bleed + Tarsus	4	269.397	1.845
Expl ~ Tarsus	3	269.482	1.930
Expl ~ Date of Bleed + Year	4	269.543	1.992

Note: The fully saturated model calculated exploration scores as a function of logarithmically transformed androgen concentrations, date of bleed, sex, year, and tarsus length. All combinations of these predictors were assessed, but only models within 2 AICc units of the highest-ranked model (i.e. that with the lowest AICc value) are reported in this table, along with the null model. The most parsimonious model was identified as the simplest model within 2 AICc units of the highest-ranked (lowest AICc) model (following Mazerolle, 2006) and is identified here in bold type.

Table A.6 AICc-ranked selection table for models predicting latitudinal migration distance (inferred from $\delta^2\text{H}$) as a function of logarithmically transformed androgen concentration and other potential covariates

Model	<i>df</i>	AICc	ΔAICc
$\delta^2\text{H} \sim \text{Date of Bleed} + \text{Sex}$	4	561.982	0.000
$\delta^2\text{H} \sim \text{Date of Bleed} + \text{Sex} + \text{Year}$	5	562.467	0.486
$\delta^2\text{H} \sim \text{Date of Bleed} + \text{Sex} + \text{Age}$	5	563.602	1.620
$\delta^2\text{H} \sim \text{Date of Bleed} + \log[\text{Andr}]$	5	564.175	2.193
$\delta^2\text{H} \sim 1$ (Null Model)	2	573.181	11.199

Note: The fully saturated model calculated latitudinal migration distance (inferred from $\delta^2\text{H}$) as a function of logarithmically transformed androgen concentrations, date of bleed, sex, year, age, and tarsus length. All combinations of these predictors were assessed, but only models within 2 AICc units of the highest-ranked model (i.e. that with the lowest AICc value) are reported in this table, along with the null model. The most parsimonious model was identified as the simplest model within 2 AICc units of the highest-ranked (lowest AICc) model (following Mazerolle, 2006) and is identified here in bold type.

Table A.7 AICc-ranked selection table for models predicting total migration distance using the full dataset as a function of logarithmically transformed androgen concentration and other potential covariates

Model	<i>df</i>	AICc	Δ AICc
Distance ~ Date of Bleed	3	977.146	0.000
Distance ~ Date of Bleed + Sex	4	978.324	1.178
Distance ~ Date of Bleed + Year	4	978.363	1.218
Distance ~ Date of Bleed + Age	4	979.064	1.918
Distance ~ Date of Bleed + log[Andr]	4	979.315	2.169
Distance ~ 1 (Null Model)	2	983.437	6.292

Note: The full model calculated estimated migration distance as a function of logarithmically transformed androgen concentrations, date of bleed, sex, year, and tarsus length. All combinations of these predictors were assessed, but only models within 2 AICc units of the highest-ranked model (i.e. that with the lowest AICc value) are reported in this table, along with the null model. The most parsimonious model was identified as the simplest model within 2 AICc units of the highest-ranked (lowest AICc) model (following Mazerolle, 2006) and is identified here in bold type.

Table A.8 AICc-ranked selection table for models predicting total migration distance excluding the three northernmost values as a function of logarithmically transformed androgen concentration and other potential covariates

Model	<i>df</i>	AICc	Δ AICc
Distance ~ Date of Bleed + Sex + Age	5	856.520	0.000
Distance ~ Date of Bleed + Sex	4	856.869	0.348
Distance ~ Date of Bleed + Age	4	857.156	0.635
Distance ~ Date of Bleed + Sex + Year	5	857.707	1.186
Distance ~ Date of Bleed + Sex + Age + Year	6	858.223	1.703
Distance ~ Date of Bleed + log[Andr] + Age	5	858.283	1.763
Distance ~ 1 (Null Model)	2	863.031	6.511

Note: The full model calculated estimated migration distance excluding the three northernmost values as a function of logarithmically transformed androgen concentrations, date of bleed, sex, year, and tarsus length. All combinations of these predictors were assessed, but only models within 2 AICc units of the highest-ranked model (i.e. that with the lowest AICc value) are reported in this table, along with the null model. The most parsimonious model was identified as the simplest model within 2 AICc units of the highest-ranked (lowest AICc) model (following Mazerolle, 2006) and is identified here in bold type.

7. *Appendix B*

All studies followed the ethical guidelines from the Canadian Council on Animals Care, which was reviewed and approved by the Animal Use Subcommittee (AUS) at the University of Western Ontario. Below is the approval email from the AUS. Animal Use Protocol #: 2016-017.



AUP Number: 2016-017

PI Name: Macdougall-Shackleton, Elizabeth

AUP Title: Mating Signals, Gene Flow, and Disease Resistance in Songbirds

Official Notification of ACC Approval: A MODIFICATION to Animal Use Protocol **2016-017** has been approved.

Please at this time review your AUP with your research team to ensure full understanding by everyone listed within this AUP.

As per your declaration within this approved AUP, you are obligated to ensure that:

1) Animals used in this research project will be cared for in alignment with:

a) Western's Senate MAPPs 7.12, 7.10, and 7.15

http://www.uwo.ca/univsec/policies_procedures/research.html

b) University Council on Animal Care Policies and related Animal Care Committee procedures

c)

http://uwo.ca/research/services/animalethics/animal_care_and_use_policies.htm

2) As per UCAC's Animal Use Protocols Policy,

a) this AUP accurately represents intended animal use;

b) external approvals associated with this AUP,

including permits and scientific/departmental peer approvals, are complete and accurate;

c) any divergence from this AUP will not be undertaken until the related Protocol Modification is approved by the ACC; and

d) AUP form submissions - Annual Protocol Renewals

and Full AUP Renewals - will be submitted and attended to within timeframes outlined by the

ACC. http://uwo.ca/research/services/animalethics/animal_use_protocols.html

3) As per MAPP 7.10 all individuals listed within this AUP as having any hands-on animal contact will

a) be made familiar with and have direct access to this

AUP;

b) complete all required CCAC mandatory training

(training@uwo.ca); and

c) be overseen by me to ensure appropriate care and

use of animals.

4) As per MAPP 7.15,

a) Practice will align with approved AUP elements;

b) Unrestricted access to all animal areas will be given to ACVS Veterinarians and ACC Leaders;

c) UCAC policies and related ACC procedures will be followed, including but not limited to:

- i) Research Animal Procurement
- ii) Animal Care and Use Records
- iii) Sick Animal Response
- iv) Continuing Care Visits

5) As per institutional OH&S policies, all individuals listed within this AUP who will be using or potentially exposed to hazardous materials will have completed in advance the appropriate institutional OH&S training, facility-level training, and reviewed related (M)SDS Sheets,

<http://www.uwo.ca/hr/learning/required/index.html>

Submitted by: Copeman, Laura
on behalf of the Animal Care Committee
University Council on Animal Care

The University of Western Ontario
Animal Care Committee / University Council on Animal Care
London, Ontario Canada N6A 5C1
519-661-2111 x 88792 Fax 519-661-2028
[auspc@uwo.ca]auspc@uwo.ca
<http://www.uwo.ca/research/services/animalethics/index.html>

8. *Curriculum Vitae*

EDUCATION

Bachelor of Science, Honours Specialization in Biology (2017) Western University, London, ON

AWARDS AND SCHOLARSHIPS

Helen I. Battle Medal and Scholarship in Zoology and Animal Biology (2017)

Dean's Honor List, Faculty of Science, University of Western Ontario (2016 & 2017)

Martin Gerardus Strybosch Fellowship Scholarship, University of Western Ontario (2013)

TEACHING APPOINTMENTS

Teaching assistant: Bio 1002 - Biology for Science II, Western University, Winter 2020.

Teaching assistant: Bio 3436 – Animal Behaviour, Western University, Fall 2019.

Teaching assistant: Bio 3484 – Patterns in the Diversity of Life, Western University, Winter 2019.

Teaching assistant: Bio 1001 - Biology for Science I, Western University, Fall 2018.

Teaching assistant: Bio 3436 – Animal Behaviour, Western University, Fall 2018.

Teaching assistant: Bio 3484 - Patterns in the Diversity of Life, Western University, Winter 2018.

CONTRIBUTIONS TO RESEARCH

Posliff, C., and Elizabeth MacDougall-Shackleton. “Consistency of movement behaviour over multiple scales in song sparrows”. Oral Presentation, Animal Behaviour Society Conference, University of Illinois Chicago, Chicago, Illinois, USA, July 2019.