American Society of Naturalists Address

What Do Ecology, Evolution, and Behavior Have in Common?
The Organism in the Middle*

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Abstract: Biologists who publish in The American Naturalist are drawn to its unifying mission of covering research in the fields of ecology, evolution, behavior, and integrative biology. Presented here is one scientist’s attempt to straddle these fields by focusing on a single organism. It is also an account of how time spent in the field stimulates a naturalist to wonder “why did that animal just do that?” and how research is guided by chance and intention interacting with the scientific literature and the people one meets along the way. With respect to the science, the examples come from bird migration, hormones and their connection to phenotypic integration, sexual and natural selection, and urban ecology. They also come from research on the impact of environmental change on the timing of reproduction and the potential for allochrony in migratory species to influence population divergence.

Keywords: seasonality, environmental change, plasticity, allochrony, heteropatry, population divergence, junco.

Introduction

Those who serve as president of the American Society of Naturalists are granted the opportunity to speak at the annual meeting. In my case, the meeting was held in Brazil in 2015, so I was triply blessed. I had the opportunity to visit a beautiful country, speak before my colleagues, and prepare this address for publication in The American Naturalist.

In Brazil, I spoke about the research I currently pursue, which addresses behavioral and physiological mechanisms regulating the seasonal timing of migration in birds in relation to environmental change and population divergence. In preparing to write, I was prompted to think about how the study of migration has advanced during the time I have been a scientist. As a young scientist, I was entranced by migration biology, but I drifted away, partially because of the lack of tools that are now in common use. These newly developed tools include stable isotopes, which allow us to determine where an animal was when it grew a feather; tracking devices, which allow us to connect an animal’s breeding and wintering ranges; and molecular tools, which allow us to detect fine-scale geographic variation in population structure. These tools and the accompanying explosion of knowledge drew me back to migration science in a way that I hope is informed by topics I studied in the interim: part ecology, part evolution, and part animal behavior.

Thus, as I prepared to write, I became more aware of how my history as a biologist led me to the title of this contribution, “What Do Ecology, Evolution, and Behavior Have in Common? The Organism in the Middle.” Perhaps sharing this history will prove useful to those who wonder where their science might lead them.

We often tell students to focus on questions, not organisms. Once the question and hypotheses are clear, the best system for testing ideas will also be clear. Many of my admired colleagues focus on specific questions, such as why organisms reproduce sexually when asexual reproduction would seem to provide greater returns, how plant mating systems evolve, or the role of female-female competition in sexual selection. Their contributions impress me deeply. My path has been different. I formed an attachment to an organism, and have followed where it led. The result has been a consistent quest for understanding that combines proximate and ultimate explanations for why animals do what they do (Mayr 1961). The organism is a north-temperate sparrow called the dark-eyed junco (Junco hyemalis): it weighs about 22 g, or about 1 ounce for those who use the English system. It is gray and white, sometimes reddish brown, and regularly visits bird feeders (Nolan et al. 2002). It flocks in winter and is socially monogamous and territorial when breeding. Its history and
the history of people who have studied it were recently summarized in a book published by the University of Chicago Press (Ketterson and Atwell 2016) and in a science documentary film (https://vimeo.com/user3004053).

Thus, in part this is an account of how my interest in this bird has fueled my research, causing me to move from one question to another while the bird remained the same. Some of these moves were inspired by the authors of an article I read, others by colleagues who entered my department, collaborators who challenged my thinking, and certainly students and postdocs who joined my lab. The most influential person was Val Nolan, who was my collaborator and partner for 40 years. Others who have inspired me especially are science friends and collaborators Ela Hau, Borja Milá, Patty Parker, Trevor Price, and John Wingfield. Last, I acknowledge the influence of National Science Foundation’s requirement of broader impacts and the galvanizing impact of the ecological and social challenges posed by environmental change to awaken a strong desire to conduct science that is both basic and applied.

The Junco
In brief, juncos are a classic organism for studying photoperiodism (Rowan 1931) and speciation (Miller 1941). The number of officially recognized species comprising the genus has fluctuated over time (Ketterson and Atwell 2016). Currently the total is five species and multiple subspecies or forms found in Central and North America. The northern dark-eyed junco (Junco hyemalis), which is the most diverse in appearance and broadest in distribution, consists of 8–10 recognizable phenotypes, at least six of which are readily recognizable in nature by plumage and at least seven of which are distinguishable genetically (Friis et al. 2016). These subspecies or forms presently occupy virtually nonoverlapping breeding ranges in North America. Some distributions are large, ranging from New England to Alaska (slate-colored junco); others are quite local, limited to sky islands in western North America (white-winged junco, pink-sided junco, gray-headed junco, and red-backed junco). The locations of these forms during the last glacial maximum have been debated (Milá et al. 2016; Price and Hooper 2016), but the strongest evidence suggests that present-day forms were confined to Mesoamerica and/or Alaska and have since expanded their ranges across the continent and diverged genetically during the past 15,000 years (Milá et al. 2016; Friis et al. 2018).

Current levels of genetic divergence in the northern forms are quite shallow. On the basis of mitochondrial DNA they were a polytomy, and only recently has their phylogeny been resolved using single-nucleotide polymorphisms and genotype-by-sequencing techniques (Friis et al. 2016, 2018; fig. 1). Despite their genetic similarities, these forms currently look and behave differently across their range in terms of morphology, migration patterns, and breeding phenology, making them a striking example of evolution in action. A recent study, for example, revealed that the plumage differences in two subspecies are literally skin deep, more a function of gene expression than differences in gene sequence (Abolins-Abols et al. 2018).

Migration
Differential Migration by Sex and Age
In my first years as a scientist I took an integrative approach, focusing on population biology, physiology, and behavior to explain variation in migratory behavior. My advisor, Val Nolan, had observed that the sex ratio of winter populations of juncos in the midwestern United States (Indiana) was male biased. That bias could have been explained by sexual segregation based on habitat or, on a larger geographic scale, by latitude. By studying museum collections, sampling junco populations in the wild, and interpreting variation in sex ratio in light of geographic variation in abundance (as determined by citizen scientists participating in Christmas Bird Counts), we were able to show that male juncos outnumbered females at higher latitudes and that female juncos were more abundant than males at lower latitudes. Assuming a more or less equal sex ratio in the breeding range, we concluded that females were making longer migrations than males, a pattern we referred to as differential migration (Ketterson and Nolan 1976, 1983). We were not the first to describe sex differences in migratory behavior; as with so many interesting questions, David Lack had done so earlier (Lack 1944). We speculated as to why the sexes might differ in distance migrated and considered three hypotheses: aggressive males might force females into longer migrations (the dominance hypothesis), stiffer sexual selection on males to arrive early at breeding sites might favor shorter migrations by males (the early-arrival hypothesis), and lesser capacity of females to endure fasting during winter storms owing to their somewhat smaller body size might favor longer migrations by females to regions where winters are milder (the body size hypothesis; Ketterson and Nolan 1976, 1983). We also proposed a multifactor hypothesis that added the cost of migration. Recent research by others on differential migration continues to sift the degree to which these explanations for differential migration apply to other species (Gow and Wiebe 2014; MacDonald et al. 2016; Deakin et al. 2019) and to generate new hypotheses, such as thermal tolerance (Alonzo et al. 2009) and differential energy requirements (Hedh and Hedenstrom 2019).

In terms of mechanisms, we studied differences in indices of migratory behavior exhibited by captive males...
and females in a common-garden environment (fattening and Zugunruhe; Ketterson and Nolan 1985). We predicted that if migratory distance were programmed, females in autumn would exhibit more activity per night or a greater number of active nights than males. We found strong differences between first-time migrants and adults, but the evidence for sex differences was weak. I also asked whether males were dominant to females when foraging in winter (Ketterson 1979; yes) and whether males were better prepared physiologically than females to endure fasting (Ketterson

Figure 1: Geographic distribution of phenotypic variation and phylogenetic relationships among junco forms. Shown is a distribution map of the different junco forms. Colored areas correspond to the breeding ranges of the major forms (ORJU = Oregon junco; PSJU = pink-sided junco; GUJU = Guadalupe junco; BAJU = Baja junco; WWJU = white-winged junco; GHJU = gray-headed junco; RBJU = red-backed junco; YEJU = yellow-eyed junco; GTJU = Guatemala junco; VOJU = volcano junco; SCJU = slate-colored junco), and points depict isolated localities with hybrid/intermediate individuals. From Friis et al. (2016).
and King 1977; yes). Together, the answers supported a multivariate explanation for differential migration (Ketterson and Nolan 1983).

For me, the takeaway message was that sex differences assume many forms and that events during the nonbreeding season are just as important to understand as those associated with reproduction. New knowledge is coming to bear on the potential implications of differential migration for conservation. Given partial sexual segregation during winter, if habitat loss were to occur in a only portion of a species’ winter range, it could lead to a biased sex ratio in the breeding range (Atwell et al. 2011; Fudickar and Ketterson 2018) or to one sex arriving in poorer condition after spring migration (Akresh et al. 2019).

Site Fidelity

Our migration studies moved in a different direction, focusing on the phenomenon of site fidelity and, as before, the interaction of genes and environment in behavior. There is something amazing about the ability of a bird to find its way home. How does a bird do that, and how does it know when to stop? If returning to a familiar place, how does it recognize its destination? Annual return to specific breeding sites by migratory birds is common, but fidelity to winter sites is less so, at least in species that are not territorial in winter (Holmes et al. 1989; Somersho et al. 2009). We pursued a series of studies in which we translocated birds to different locations to assess the impact of prior experience on migratory behavior and site recognition. We asked whether individuals that were transported to or held at their migratory destination of a prior year would suppress their programmed motivation to migrate owing to site recognition (Ketterson and Nolan 1986, 1987; Sniegowski et al. 2010). Results of these studies involved juncos and indigo buntings and suggested that individuals recognized their destination (site recognition), which suppressed normal migratory behavior. However, the effects were small, so I cannot say that they were conclusive. In general, these questions are still unanswered.

As it turned out, some of these studies involved manipulations of day length with a focus on seasonality (Nolan and Ketterson 1990) and led our research into the domain of annual cycles and regulation of migration and reproduction. They also introduced me to several of the scientists studying migration in Germany who had a big impact on my thinking, for example, Ebo Gwinner and Peter Berthold. They were strong proponents of genetically determined migratory behavior, but because Nolan and I had found that experience plays an important role in migratory behavior, it seemed that genetic determinism of migratory timing might be overemphasized (but see Biebach 1983). The relationship between site fidelity and seasonal migration remains a topic of current interest and may account for some cases of static geographic distributions and inflexible migratory routes despite recent environmental change (Winger et al. 2019).

People currently studying migratory species emphasize the importance of studying the full annual cycle (Marra et al. 2015). An important next step to studying differential migration in the 1980s would have been to connect wintering birds to their breeding locations, but the ability to do that was far off in the future (Briedis and Bauer 2018). Another approach would have been to study heritability of migratory behavior, but most songbirds are ill suited for genetic studies because so few breed successfully in captivity (but see Pulido et al. 1996). In addition, at the time natural pedigrees were few, limiting the impact of quantitative genetics (Van Noordwijk 1984), although this has changed markedly. Finally, molecular tools for assessing the genetic structure of natural populations and mapping migratory patterns were barely underway and primarily useful for highly divergent forms only (species and genera or orders; Shields and Straus 1975). There were no tags for tracking individuals to determine migratory connectivity (Rushing et al. 2014) or stable isotopes to determine where migrants on their winter range might have originated, as can now be done (Hobson 1999; Steenweg et al. 2017; Brattstrom et al. 2018). Our attention therefore turned in another direction.

Mating Systems, Trade-Offs, and Phenotypic Integration

Mating systems were a topic of great interest in the 1980s, and the topic appealed to my combined interest in “how questions” as well as “why questions.” About this time, I read an article by John Wingfield titled “Androgens and Mating Systems: Testosterone-Induced Polygyny in Normally Monogamous Birds” (Wingfield 1984) that turned my research in a new direction. I asked Wingfield if I might visit his lab at the Rockefeller Field Research Center to learn more about the role of testosterone in mating systems and parental care. He graciously hosted me, and he and Marilyn Ramenofsky shared their methods, their thinking, and the company of John’s postdocs at the time, Al Dufty, Bob Hegner, and Greg Ball. They gave me the opportunity to apply the knowledge gained to research on the junco.

Juncos do not breed in Indiana, where I lived, but there are US populations that breed in the Appalachian Mountains of eastern North America and are year-round residents. Nolan and I studied them at Mountain Lake
Biological Station. Stimulated by Hegner and Wingfield (1987), we took an experimental approach to the evolution of mating systems we called “phenotypic engineering.” We used silastic implants to experimentally elevate testosterone in males and compared them to controls for phenotype and fitness (Ketterson et al. 1996). When this research was just beginning, Mary Jane West-Eberhard visited Mountain Lake Biological Station. When I described it to her, she said it was important, greatly boosting my confidence. I remain grateful.

We began this work on the basis of prior research showing that experimentally elevated testosterone induced polygyny and suppressed parental behavior. We predicted that females mated to testosterone-treated males might “enforce” parental behavior by deserting males that were unreliable. While sitting near a nest counting parental visits with food early in this research, I noticed that the male perched above the nest was not feeding but was singing extremely often. It dawned on me that the hormone had affected more than the trait of parental behavior. That particular bird served as my inspiration for what came next.

We went on to learn that testosterone altered multiple aspects of male physiology and behavior—for example, enhancing home range size, male attractiveness to females, and response to stressors while reducing parental behavior (Ketterson et al. 1992; Chandler et al. 1994; Enstrom et al. 1997; Ketterson and Nolan 1999; Schoech et al. 1999). Males treated with testosterone did not become polygynous, as we predicted, but they did achieve greater reproductive success via extrapair fertilizations (Raouf et al. 1997; Reed et al. 2006). That success, however, was accompanied by reduced survival (Reed et al. 2006), a clear demonstration of a life-history trade-off. Phenotypic engineering is an approach still taken today, including cases where other investigators may have proposed it independently (Lee et al. 2008; Nugent et al. 2019).

I believe the most important takeaway from these studies was finding that manipulating a single molecule in a bird’s systemic circulation could alter the expression of multiple behavioral, physiological, and morphological traits. We termed this “hormonal pleiotropy,” a concept that is still in use (McGlothlin and Ketterson 2016; Dantzer and Swanson 2017; Bourg et al. 2019), as is a closely related and more general term, “phenotypic integration” (Pigliucci 2003).

An implication of hormonal pleiotropy is that a change in the environment that favors one hormone-mediated trait might also select for correlated traits when they share a common mechanism. If those traits are also advantageous, adaptation might proceed swiftly. But if the correlated traits are disadvantageous, a hormonal change might act as a constraint, at least temporarily (Ketterson and Nolan 1999; Ketterson et al. 2009). This balance between adaptation and constraint is highly relevant to how rapidly organisms can respond to environmental change. It is also relevant to the concepts of phenotypic integration and phenotypic independence, which can be seen as a balance between systemic hormonal signals that favor coordinated trait expression and tissue-specific hormone sensitivity that allows for independent expression of genes and cellular processes regulated by locally produced steroids (Rosvall et al. 2016). That is, hormones can generate integration or permit independence, depending on how they influence the traits that comprise the organism (Bergeon Burns et al. 2013).

In a recent article, Bourg and colleagues modeled the genetics of endocrine-mediated trade-offs, concluding that when energy is sufficient, constraints can be overcome because there are hormonal mechanisms in place to foster the allocation of “extra energy” to support multiple, ordinarily competing traits (Bourg et al. 2019). Lofeu and colleagues recently attributed coordinated trait expression in developing tadpoles to hormonal-mediated phenotypic integration (Lofeu et al. 2017). Another recent contribution to issues surrounding hormonal pleiotropy and constraint addressed the neck muscle that allows woodpeckers to drum on tree trunks and is stimulated by androgens. Schuppe and Fuxjager (2019) concluded that circulating concentrations of testosterone and an abundance of androgen receptors may be less constraining to muscle development than the nuclear receptor cofactors that follow cellular receipt of a hormone and lead to activation of gene expression.

The research in Virginia expanded as we extended the experimental treatment with testosterone to females, asking whether male evolution might be constrained by sexual conflict if elevated testosterone were disadvantageous to females, which it turned out to be (Ketterson et al. 2005; O’Neal et al. 2008; Gerlach and Ketterson 2013). We also asked whether selection on natural variation in male testosterone would mirror the experimental results seen in males, and in some but not all ways, the answer was yes. For example, as had been true of testosterone-treated males, males with naturally high levels of testosterone showed lower survival (McGlothlin et al. 2007). We also asked how experimentally elevated testosterone altered gene expression in males and females in order to assess the potential for sexual conflict (Peterson et al. 2013, 2014). An indirect consequence of the many years spent at Mountain Lake Biological Station was the opportunity to observe cycling in junco abundance in inverse relation to acorns and rodents (Clotfelter et al. 2007), to detect heritable variation in success at extrapair copulations (Gerlach et al. 2012) and to measure the impact of dispersal on genetic structure (Liebgold et al. 2013; Liebgold et al. 2019).
Hormones and Rapid Response to a Novel Environment

A chance meeting with Trevor Price at a National Science Foundation workshop led me to wonder whether what we had learned about hormones and phenotypes might apply to a case of rapid evolution by juncos that had recently colonized an urban environment in Southern California. Price, his student Pamela Yeh, and others in their group had reported that juncos that formerly lived on the coast of Southern California only during winter and departed in spring had ceased to migrate and begun to breed in San Diego (Yeh 2004; Yeh and Price 2004; Price et al. 2008). Compared with a nearby migratory, nonurban, and montane population, the colonists bred earlier in the year, were less aggressive, and were less strikingly ornamented (Rasner et al. 2004). The plumage difference (less white on tail feathers) persisted when nestlings of both populations were raised in a common garden (Yeh 2004). We noted that the traits exhibited by colonists in response to a novel environment resembled those that could potentially result from a reduction in circulating levels of testosterone. We asked whether a change in testosterone played a role in this rapid genetic divergence.

Jonathan Atwell, then a graduate student at Indiana University, found that colonists indeed had lower testosterone levels than migrants when breeding and that they exhibited other traits associated with reduced testosterone, such as greater investment in parental behavior (Atwell et al. 2012; fig. 2). Atwell also found that urban birds were bolder and less likely to elevate the stress hormone corticosterone when handled (Atwell et al. 2012). Some of the traits that differed between wild populations persisted in a common garden, such as differences in vocal behavior (D. G. Reichard, J. W. Atwell, M. M. Pandit, G. C. Cardoso, T. D. Price, and E. D. Ketterson, unpublished manuscript) and response to stressors (Atwell et al. 2012), but the difference in testosterone did not (Atwell et al. 2014). Thus, some but not all of the predictions that hormones might facilitate rapid adjustments in correlated phenotypic traits in response to a novel environment were supported. Whether the colonization should be considered an invasion or an adaptation to urban environments is an open question. Another unanswered question is how the colonists became sedentary over such a short period of time. What were the mechanisms behind the rapid loss of migratory behavior and earlier reproduction that accompanied colonization, and to what degree was (and is) their divergence a function of drift and selection (Frisse et al. 2018)?

At about this time, it became apparent that many of the questions about migration that had been unanswerable in 1980s were now addressable given advances in technology. The barriers to the study of migration were falling away. Furthermore, much had been learned about the brain, behavior, and the regulation of seasonal timing.

Hormones, Migration, and Population Divergence

A recent shift in the research questions I address began when I read an article by Kevin Winker, who is an ornithologist and evolutionary biologist (Winker 2010). Winker devised a new term, “heteropatry,” to describe population distributions. Winker noted that many closely related avian populations live in sympatry for part of the year, but because some migrate and some do not, they become allopatric when breeding, that is, their distribution is heteropatric. Winker made a set of predictions about how migration-related heteropatry might foster population divergence. He proposed that selection might act against migrant-resident hybrids if hybrids exhibited a mismatch in their seasonal timing to the local environment. He speculated that because resident populations often breed earlier than migratory populations, a migrant-resident hybrid that migrated might breed too early for the climate where it settled, with a concomitant reduction in relative fitness. Conversely, a migrant-resident hybrid that did not migrate might breed later than its conspecifics and suffer the fitness disadvantage often associated with later-produced offspring (Price et al. 1988; but see Graham et al. 2019). In Winker’s view, migration and timing of reproduction are tightly linked; when the match is poor, the consequences are negative. This view of migratory timing brings to mind the concept of a magic trait, defined as one that contributes to divergence in sympatry (Servedio et al. 2011).

Winker also predicted that early stages of heteropatric speciation might first be associated with altered gene

![Figure 2: Hormones can have pleiotropic effects on suites of traits, enhancing some (plus signs) and suppressing others (minus sign). Those noted here are based on prior experimental (boldface) and correlational (italics) results from the junco, as reviewed in Ketterson et al. (2009). When populations encounter novel or changing environments, such hormonal signals may shape multitrait responses. From Atwell et al. (2014).](image-url)
expression, as opposed to mutation, followed by genetic accommodation, a prediction that received recent support (Saino et al. 2017). Winker further suggested that selection against hybrids might reinforce divergence not just in timing. Differences in appearance might be favored if they served as signals revealing whether a close relative would be likely to share timing traits and thus make an appropriate mate. To invoke his own words, Winker (2010, p. 19) wrote the following:

Prediction 6.—Finally, in considering mechanisms that initiate the process [divergence] and that enable differentiation to progress under this model, it is possible that developmental (or phenotypic) plasticity and variation in gene expression are more important than mutation, at least in early phases. Subsequently, selection (which acts on the phenotype) causes gene frequency changes through genetic accommodation that are associated with the favored traits (Berthold 2003; Price et al. 2003; Rappole et al. 2003; West-Eberhard 2003; West-Eberhard 2005). Under this scenario, cyclic behaviors are induced by environmental inputs (environmental induction; West-Eberhard 2003, 2005). This results in a local behavioral response—increase movement with a go-and-return component—to exploit local (i.e., nearby) resource peaks that are different in space and time from those available to individuals that do not undertake such movements. If favorable, these new phenotypes result in an increased frequency of genes associated with the new behavioral phenotype(s). Then, through such genetic accommodation, genes that regulate the associated behaviors and developmental thresholds (e.g., in timing of gonadal growth and recrudescence, fattening, molt, territorial behavior, mate selection, and song) “lock in” an endogenous direction (anagenesis) to cyclic migration and the process of differentiation (cladogenesis) in relation to sub-populations that do not exhibit such movements (or exhibit the traits on a different evolutionary trajectory).

To a person accustomed to thinking about migration, annual cycles, hormonal pleiotropy, mating preferences, and phenotypic integration, Winker’s words had a powerful impact, and I became deeply curious about how they might apply to the junco’s diversification.

Winker is not alone in having related differences in seasonal timing to population divergence. Students of sympatric speciation have long written about the potential for timing differences, “allochrony,” to create circumstances favorable to divergence, perhaps more often in plants than in animals (Skeels and Cardillo 2019). The concept most often employed when relating migratory behavior to sympatric speciation in birds is that of migratory divides (Price 2008; Taylor and Friesen 2017; Turbek et al. 2018). Divides refer to the situation where closely related populations have overlapping breeding ranges but separate wintering ranges (or migratory routes). These differences can lead to asynchronous return times to the breeding site that result in assortative mating based on migratory timing (Helbig 1991). A now-classic example of a migratory divide and divergence is the blackcap (Sylvia atricapilla), an Old World warbler (Rolshausen et al. 2010).

Connections between timing mechanisms and population divergence are receiving increased emphasis (Rolshausen et al. 2009; Helm et al. 2013; Helm and Womack 2018), although references to Winker’s important ideas remain few. A difference to note between Winker’s heteropatry and the literature on migratory divides (Turbek et al. 2018) is that Winker focused on populations whose wintering ranges overlap. That is, unlike the case with migratory divides, where populations are sympatric when breeding, Winker emphasized situations in which populations are sympatric during the nonbreeding season and allopatric when breeding because some migrate and some do not. Winker’s ideas also emphasize historical interactions when asking how sympatric nonbreeding populations become allopatric when breeding. Might the process resemble the repopulation of regions that were unsuitable for breeding at the glacial maxima? How might such dynamics play out under current environmental change?

My experience with migratory and resident juncos in the Appalachian Mountains of Virginia did not involve migratory divides. Migratory juncos are present in Virginia in winter, but most depart for higher latitudes by the time the residents reproduce (fig. 3). The reason for lack of hybridization between migrants and residents seemed clear. Or was it? What if migrant males produced sperm before departing and copulated with residents? How long did sperm survive in the female’s reproductive tract? Might there be undetected gene flow to discover? Furthermore, just because migrants appeared not to overlap when reproducing in the present, had it always been that way? Where were these juncos during the Pleistocene? And where will they be as the climate changes?

In short, I found Winker’s predictions provocative when applied to the junco and resolved to learn more about what stimulates migration in migrants and the mechanisms that regulate the timing of reproduction. To make predictions about responses to environmental change, it would be necessary to learn whether differences in behavior and timing were fixed as a result of differences in gene sequence, as local adaptation might predict, or more flexible, perhaps due to variation in gene expression with an epigenetic basis and rapidly reversible. Also important would be to learn more about mate choice and whether migratory and
resident juncos would still mate assortatively, even if they were sympatric.

I teamed up with Tim Greives, who is an expert in seasonality, to address how neuroendocrine mechanisms (brain and hormones) that influence seasonal timing of migration and reproduction might differ between migrant and resident juncos as well as to address whether the answer might depend on how much time had passed since migrants and residents had become heteropatric. We hoped the answers might apply broadly to how vertebrate populations may respond to environmental change and also relate to whether changes in the selective landscape might lead to gains or losses in biodiversity. Soon joined by Adam Fudickar and Abby Kimmitt working with Ketterson and Carolyn Bauer, Jessica Graham, and Katie Needham working with Greives, we followed a path that was familiar—to integrate mechanism and function to seek greater understanding of how organisms will adjust to global change.

**Natural History of Seasonal Timing of Reproduction in Birds**

Birds and most other animals that live in seasonal environments typically breed during the season when food is most abundant for provisioning offspring. Between bouts of reproduction, birds regress their reproductive organs. Some migrate and avoid the cold and short days of winter found at higher latitudes. In spring, reproductive development resumes and is regulated by a region of the brain, the hypothalamus (H), which perceives changes in day length and other environmental cues and communicates that information via hormones to tissues found in other parts of the body, including the pituitary gland (P) and the gonad (G). Collectively, they are known as the HPG axis, whose response to the environment culminates in renewed gonadal growth and preparation to reproduce and fosters phenotypic integration (fig. 3). Some cues and signals accelerate gonadal growth; others delay growth and development. This balance between acceleration and delay determines the timing of reproduction. When things go well, the outcome is to match reproductive timing to a given environment and thus to maximize the likelihood of reproducing successfully. Another endocrine axis, the hypothalamic-pituitary-adrenal axis (HPA axis), supports metabolic responses to stressors in the environment and can in some cases suppress reproduction until the stressors have passed. While the hormonal basis for reproductive timing has long been a subject of intense study, the hormonal basis for migration—and the transition from migration to reproduction—is less well understood, although Marilyn Ramenofsky and colleagues have made significant progress (Ramenofsky and Wingfield 2017; Ramenofsky et al. 2017).

**Mechanisms of Reproductive and Migratory Timing in the Junco**

The power of the junco system for studying timing mechanisms is that closely related resident and migrant junco populations live together prior to spring departure by migrants and are thus exposed to the same environment (fig. 4). If similar cues lead to different responses, what is the internal cause? Are behavioral and physiological differences between migrants and residents fixed or flexible? Are timing differences suppressing gene flow and supporting population divergence? And as the environmental changes, will the mechanisms foster or constrain adaptation?

**Migrant and Resident Timing in a Common Garden**

To see whether timing differences between migrants and residents were fixed, we captured members of both populations in Virginia, housed them together, and followed their reproductive development the following spring, when they were provided with the same food and temperature and exposed to natural increases in day length. We learned that migrants fattened, but residents did not. In contrast,
Figure 4: Seasonal variation in distribution and events of the annual cycle of seasonally sympatric migratory and sedentary dark-eyed juncos (*Junco hyemalis*). After fall migration, migratory and sedentary juncos mix at shared wintering habitats (shaded blue). In early spring, while still in sympatry, sedentary juncos begin preparations to breed while migrants prepare for migration despite exposure to identical environmental cues. From Fudickar et al. (2016a). Image prepared by Jonathan Atwell.
residents became reproductive earlier, as revealed by elevations in testosterone in response to injection with gonadotropin-releasing hormone (GnRH; Fudickar et al. 2016a; fig. 5A). Delayed reproductive development in migrants is consistent with a potential role for timing in suppressing gene flow. Interestingly, however, the differences between migrant and resident populations in the timing of reproductive development (as measured by gonad mass) were not categorical but clinal. That is, within migrant males, individuals that made longer migrations, as estimated by stable isotopes of hydrogen, had smaller testes on a given date than did individuals that made shorter migrations (Fudickar et al. 2016a; fig. 5B). We recently replicated this result (Singh et al. 2019) and extended the finding to females (Kimmitt et al. 2019). We were surprised and fascinated to find this continuous variation in reproductive timing in relation to breeding location, which seems to indicate local adaptation based on cues received from a distance. The implications for potential fitness costs of hybridization are not yet clear.

**Negative Feedback**

To learn what accounted for delay in migrants, colleague Tim Greives hypothesized that the hypothalamus and/or pituitary, known to be the source of stimulation for gonadal growth, might also suppress reproductive development, given their sensitivity to negative feedback, and that negative feedback might be stronger in migrants than in residents, accounting for their delayed reproductive development (Greives et al. 2016). That is, the hypothalamus is a self-regulating region of the brain that indirectly stimulates testosterone production but is also suppressed by the testosterone it stimulates, much like a thermostat turns off the furnace when it gets too warm.

To test for relative suppression by negative feedback in free-living migrants and residents in the field, we injected males and females multiple times with GnRH and measured luteinizing hormone (LH). We predicted that if delayed reproductive development in migrants is a result of stronger negative feedback (suppressive effect of sex steroids on the hypothalamus), then pituitary release of LH would decline after multiple injections and do so to a greater degree in migrants. This prediction was not upheld in males but was supported in migrant females (Greives et al. 2016).

We also compared captive migrants and residents from Virginia for differences in abundance of transcripts for candidate genes related to reproduction and migration in two tissues using quantitative polymerase chain reaction. In the hypothalamus, we found that residents in Virginia had enhanced messenger RNA expression of GnRH, confirming the role of the hypothalamus in stimulation of gonadal growth. We also found decreased expression of hypothalamic androgen receptor and estrogen receptor α in residents. These findings identify increased GnRH production and decreased hypothalamic sensitivity to sex steroid negative feedback as potentially critical factors.
promoting differences in the timing of reproductive development (Bauer et al. 2018) and thus as potential targets for selection on timing as the environment changes.

**HPA Axis and Timing**

With respect to the HPA axis, which mediates the release of corticosterone (which in turn mobilizes energy and can suppress reproduction), we asked whether migrants would have higher baseline corticosterone, higher stress-induced corticosterone, and weaker negative feedback than residents. These predictions were not upheld. Baseline and stress-induced corticosterone were higher, not lower, in residents than in migrants, and there was no difference in negative feedback (Bauer et al. 2016). We concluded that the HPA axis was not suppressing early reproductive development in migrants (cf. Ramenofsky et al. 2017).

**Gene Expression**

Using RNA sequencing, we compared the migrants and residents from Virginia for differential gene expression in two tissues, muscle and blood. We identified 547 differentially expressed genes; those involved in lipid transport and metabolism were more highly expressed in migrants, and those involved in reproductive processes were more highly expressed in residents (Fudickar et al. 2016b; fig. 6). These genes serve as likely candidates for divergence in sequence and expression and will stimulate future studies. If each plays a role, then regulation of timing is polygenic. But their differential expression could also be the outcome of a switch that differs by migratory strategy (West-Eberhard 1989), in which case the transition from a migratory to a sedentary strategy, or vice versa, could be achieved rapidly.

**Time since Divergence**

The population in California that had colonized San Diego and become sedentary and that was studied by Price, Yeh, and Atwell (see above) presented the opportunity to study resident and migratory populations that differed in how long ago they had diverged. Our specific prediction was that the recently diverged resident and migratory juncos from California would be more flexible in reproductive timing and migratory tendency when held in a common garden than were the longer-diverged juncos from Virginia. This prediction was partially upheld. When juveniles captured from the resident and migratory populations in California were later compared in a common garden, their reproductive timing, as measured by GnRH-induced elevations in testosterone, was similar (i.e., flexible; Atwell et al. 2014). However, they differed in whether they

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Figure 6: Heat maps comparing migrant and resident juncos housed during spring in a common garden. Genes in muscle (A; 366 genes) and blood (B; 181 genes) were significantly differentially expressed. Each column represents an individual (blue = migrants; red = residents), and each row represents a differentially expressed gene. Expression scores were z-score normalized; darker colors represent higher expression. Dendrograms represent hierarchical clustering for visualization. From Fudickar et al. (2016b).
fattened, with only migrants fattening (i.e., fixed; J. W. Atwell, R. J. Rice, M. M. Pandit, et al., unpublished data). Furthermore, in a later study, when migrant and resident individuals were captured during winter sympatry, brought to a common garden, and compared the following spring, the recently diverged San Diego residents initiated reproductive development significantly earlier than the migrants, suggesting fixed, not flexible, differences (Fudickar et al. 2016a, 2017). The question of whether time since divergence predicts flexibility in reproductive and migratory timing remains unresolved.

**Mate Choice**

Of course timing, as a determinant of whether populations are allopatric when breeding, is only one potential cause of population divergence. Some of our most recent research has addressed whether migrants and residents would be likely to mate preferentially with each other if sympatric when breeding. Abby Kimmitt presented free-living resident males in Virginia with either a caged migrant female or a resident female and measured courtship behavior. She asked whether resident males would preferentially court resident females or show equal interest in both. She found resident males more likely to direct courtship toward resident females (Kimmitt et al. 2018). The question of how males assessed females as potential mates—that is, whether the preference was based on appearance (differences in body size, plumage, or bill color that differ between migrants and residents), stage of reproductive development (more advanced in resident females), or both—remains unanswered. One way to uncouple appearance from reproductive development would be to expose migrant females to long days to accelerate their reproductive development to match that of resident females and then repeat the mate choice trials.

**In Sum**

To summarize, reproductive development in spring proceeds as the HPG axis responds to environmental cues that culminate in gonadal growth. Output from the hypothalamus can stimulate growth; input to the hypothalamus by negative feedback can delay growth. Variation in the HPG axis between migrants and residents resides at multiple levels, some associated with stimulation and some with suppression. Variation in these mechanisms, to the best of my knowledge, reduces gene flow between migrants and residents and may contribute to incipient speciation. This conclusion is also supported by courtship preferences in free-living males.

Thus, the results of the research summarized here on mechanisms potentially relates to population divergence as envisioned by Winker by indicating how genes acting in different tissues at different times of the year influence seasonal timing and the potential for population divergence. As the environment changes, growing warmer and less predictable, gene sequences may evolve or may be turned on or off at different times of the year than they are now. Whether that will result in earlier or later departure of migrants or earlier or later timing of reproduction in residents is not known. But the answer will have consequences for the likelihood of interbreeding between migrants and residents, and the outcome could be a collapse of incipient speciation or more rapid divergence between close relatives.

**Future Directions**

Very briefly, the following research questions seem likely to bear fruit. For obvious reasons the role of female timing in population divergence deserves more attention than it has received (Kimmitt et al. 2019; Needham et al. 2019). Another open area is the degree to which differences in reproductive timing that appear to be fixed might actually be “experiential.” In some mammals photoperiodic threshold is set early in development on the basis of perceived photoperiod (Ciarleglio et al. 2011). Whether this is true of juncos is currently under investigation by Adam Fudickar.

Another unanswered question is how migratory physiology interacts with reproductive physiology—are they independent or interactive? One long-standing explanation, that activation of the HPA axis stimulates migration and suppresses reproduction, was not supported by our results. Studies underway are comparing migrants and residents for transcript abundance and methylation in the hypothalamus (D. Singh, A. M. Fudickar, C. Stricker, et al., manuscript in preparation; D. Singh, A. M. Fudickar, and E. D. Ketterson, manuscript in preparation).

Predicting future breeding distributions will be enhanced by sophisticated species distribution modeling of present-day distributions and distributions during the last glacial maximum. Much remains to be learned about whether current changes in climate have led to reduction in differential migration by sex and greater overlap in male and female distributions in winter (D. O. O’Neal and E. D. Ketterson, unpublished data). The generality of findings in Virginia can be pursued with other junco subspecies that are sympatric in winter and allopatric when breeding, several of which can be found in the southwestern United States. Of Winker’s predictions described above, our research bears on only a few of them. Big questions that remain call for the study of hybrid zones. Do traits related to timing of migration and reproduction segregate
independently, or are they linked? Do timing and mate choice covary? Does variation in timing have a genetic or epigenetic basis?

Relation to Environmental Change

All organisms reproduce, and while not all migrate, all disperse, and the processes of dispersal and migration are related. As many have noted, the degree of flexibility in seasonal timing will play an important role in determining adaptive capacity—that is, the ability of organisms to “keep up” with change (Piersma and Van Gils 2011; Quintero et al. 2014)—and concomitantly current levels of biodiversity. Among the evolutionary consequences of changes in the environment will be changes in species distributions, with accompanying gains or losses in diversity. Some populations will cease migrating, altering the likelihood of hybridization between sympatric forms that were formerly allopatric. Others may extend their migrations, giving rise to reproductive isolation between actively diverging populations that were formerly sympatric but become allopatric (Tietze 2018). Because selection on traits leads to selection on underlying mechanisms, predicting the consequences of environmental change on distribution and abundance will call for the combined insights that emerge when ecologists, evolutionary biologists, and integrative biologists bring their insights to bear on the organism.

Looking back personally? That one organism could hold a person’s attention for what has been a lifetime might suggest missed opportunities. I might have extended the study of differential migration to other species to discover why females migrate farther than males. Changing questions also creates opportunity costs. We might have continued to employ phenotypic engineering to determine how the fitness consequences of experimental elevation of testosterone vary over time or whether they are frequency dependent. Perhaps others will determine the answers. Regardless, I am certain from looking back that (1) organismal biology will play a critical role in predicting how life will respond to ongoing environmental change and (2) the American Society of Naturalists will continue to lead in the integration of ecology, evolution, and behavior.

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