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Opinion

The Role of Seasonal Migration in Population Divergence and Reproductive Isolation

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Seasonal journeys between breeding and non-breeding habitat are undertaken by a diverse array of animals. Parallel developments in tracking and genomic methods are enabling finer resolution of these movements and their role in the evolutionary process. Evidence from allopatric and co-occurring breeding populations indicates that variation in migratory behavior is often associated with genetic differentiation. While assortative mating and selection against hybrids due to divergent migratory phenotypes can contribute to reproductive isolation, the details of these mechanisms remain unclear. Here we identify gaps in our understanding of the role of seasonal migration in the speciation process and propose a framework to test the relative significance of reproductive barriers associated with variation in migratory behavior that might underlie population differentiation.

Evolutionary Outcomes of Seasonal Migration

Animal migration has evolved independently and repeatedly in a diverse array of taxonomic groups, including insects, fish, amphibians, birds, and mammals. Seasonal movements between breeding and non-breeding locations, hereafter '**seasonal migration**' (see Glossary), provide numerous ecological benefits, allowing migratory organisms to escape competition, colonize favorable habitats, and evade predators and parasites [1]. Although these seasonal journeys have captivated the human imagination for thousands of years [2], until recently studies on migratory behavior heavily relied on mark–recapture efforts to gather information on the timing of migration and migratory routes of individuals [3]. However, new innovations in tracking technology (reviewed in [3,4]), particularly the ongoing miniaturization of tracking devices (e.g., **geolocators**, satellite transmitters) and the development of indirect methods of inferring migratory behavior (e.g., **stable isotope analysis**), have created unprecedented opportunities to examine variation in migratory strategies among a wide range of taxonomic groups [5]. Combined with parallel advances in high-throughput genomic sequencing technology (see [6,7]), these developments make possible previously intractable avenues of research on the evolutionary consequences of seasonal migration, particularly the contribution of seasonal movements to the maintenance or collapse of species boundaries [8].

Seasonal migration requires an integrated suite of adaptations necessary for long-distance movement and survival in different environments [1]. As a result, migration can promote population differentiation by exposing lineages to divergent ecological conditions or limiting opportunities for genetic exchange through assortative mating or selection against hybridization [9]. Here we outline current knowledge about the role of seasonal migration in population divergence and provide a framework for studying how seasonal movements contribute to the evolution and maintenance of reproductive isolation. First, we discuss how divergent migratory behavior can lead to the evolution of differentiation between geographically isolated groups.

Highlights

The challenge of tracking migratory organisms has impeded progress in understanding the likely importance of seasonal migration in the evolution of population divergence and reproductive isolation.

Parallel advances in animal tracking and high-throughput genomic sequencing technology have opened previously intractable avenues of research on the evolutionary consequences of seasonal migration by allowing researchers to link migratory behavior to patterns of genetic exchange.

Migratory divides offer a unique opportunity for researchers to apply emerging technology to directly test the contribution of seasonal migration to reproductive isolation.

While seasonal migration may influence reproductive isolation in hybrid zones, the relative significance of reproductive barriers associated with variation in migratory behavior remains unclear.

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Then, we describe how co-occurring breeding populations that exhibit divergent migratory strategies present exciting opportunities to further our understanding of the contribution of seasonal migration to reproductive isolation.

Linking Migratory Phenotype and Population Differentiation in Allopatry

Research linking divergence in **migratory phenotype** to the speciation process has revealed that alterations in migratory behavior, combined with strong site fidelity, are often associated with genetic differentiation among closely related populations (e.g., sharks [10], sea turtles [11], warblers [12], salmon [13]). For example, Schreibers' long-fingered bat (*Miniopterus schreibersii natalensis*), which migrates between wintering roosts (hibernacula) and summer maternity colonies in South Africa, exhibits substantial genetic structure that corresponds to local habitats, wing morphology, and migratory behavior, suggesting that adaptation to local environmental conditions surrounding roosts could restrict gene flow between populations [14]. In addition, the marked segregation of mtDNA haplotypes among subpopulations of humpback whales (*Megaptera novaeanglieae*) corresponds to patterns of seasonal migration, indicating a role of maternal fidelity to discrete migratory destinations in genetic differentiation [15].

While associations between migratory behavior and genetic divergence in allopatry indicate a possible role of seasonal migration in differentiation, it is difficult to disentangle the influence of migratory phenotype on the accumulation of genetic differentiation from the confounding effects of local adaptation and geographic distance. To address these challenges, we propose indirect methods of inferring the relative influence of migratory **traits** and geographic distance on patterns of genetic divergence (Box 1). These methods will allow researchers to evaluate the potential role of divergent migratory phenotypes in population differentiation when reproductive barriers cannot be tested directly.

Linking Migratory Phenotype and Reproductive Isolation at Migratory Divides

Divergent migratory phenotypes are often associated with genetic differentiation between closely related allopatric populations [10–13]; however, we lack information about the direct contribution of divergent migratory phenotypes to reproductive isolation. **Migratory divides** provide a unique opportunity to address this gap by enabling researchers to directly test the strength of reproductive barriers linked to seasonal migration. While the term migratory divide lacks a consistent definition in the literature and has largely been restricted to avian systems, we use the phrase broadly to refer to populations that coexist during the breeding season but overwinter in separate locations (e.g., **anadromous** vs non-anadromous forms of salmonid fish [16], sedentary and migratory herds of caribou [17], wolves that prey on caribou with divergent migratory strategies [18]) or follow different migratory routes to the non-breeding grounds (e.g., beluga whales that migrate to distinct summering areas following reproduction [19], storks that take different migratory routes to bypass the Mediterranean Sea [20]). Migratory divides are formed when divergent migratory strategies that arise in allopatry are maintained on secondary contact (Figure 1A) or when divergent migratory behavior evolves *in situ* through primary divergence (Figure 1B).

In birds, migratory divides are often associated with significant genetic differentiation [21,22], formed through secondary contact [23,24], and hypothesized to directly promote and/or maintain reproductive isolation through prezygotic and post-zygotic isolating mechanisms [25]. In the case of **prezygotic reproductive isolation,** differential timing of arrival on the breeding grounds between individuals with divergent migratory phenotypes can lead to assortative mating (temporal isolation) [26]. Furthermore, selection against hybrids that

Glossary

Anadromous: a migratory strategy that involves traveling from the sea to spawning grounds in freshwater, often used in reference to fish.

Geolocator: an archival light-level logger that is used to estimate geographic locations through patterns of light intensity. Geolocators must be recovered following deployment to retrieve the data.

Migratory divide: a location where co-occurring breeding populations pursue divergent migratory strategies during the non-breeding season.

Migratory phenotype: the observable characteristics related to seasonal migration, such as migratory propensity, timing, distance, orientation, and wing morphology, that result from the interaction between an individual's genotype and its environment.

Orientation funnel: a tool used to study migratory restlessness and orientation in birds. Individuals are placed in a funnel-shaped cage and migratory activity is recorded as claw marks left on a paper spread across the cage walls.

Post-zygotic reproductive isolation: the absence or reduction of gene flow between groups due to selection against hybrids.

Prezygotic reproductive isolation: the absence or reduction of gene flow between groups due to behavioral or phenotypic differences related to the process of mate choice or assortative mating.

Reinforcement: the evolution of prezygotic reproductive barriers as a result of selection against hybrids.

Seasonal migration: the regularly timed movement of organisms between breeding and non-breeding locations occupied at different points throughout the year.

Smoltification: a series of physiological changes necessary for seaward migration in fish.

Stable isotope analysis: a method of inferring migratory behavior that leverages geographic variation in the stable isotope signatures of animal tissues. The stable isotope ratios of naturally occurring elements [e.g., carbon (C), hydrogen (H), nitrogen (N)] vary across the landscape in predictable ways and are incorporated into animal tissues

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Box 1. Demonstrating That Divergent Migratory Phenotypes Influence Genetic Differentiation among Allopatric Populations (Steps 1–3) or Prezygotic Reproductive Isolation at Migratory Divides (Steps 1–4)

This framework should be applied to closely related groups in the early stages of the speciation continuum that exhibit alternative migratory strategies.

Step 1

Document differences in migratory phenotype between breeding populations. Alternative migratory phenotypes can be detected through one or more of the following methods: stable isotopes, tracking devices, orientation funnels, recapture records, and genetic markers. In a migratory divide, individuals that differ in migratory behavior should co-occur during the breeding season.

Step 2

Assess genetic divergence between populations that differ in migratory phenotype. The level of genetic differentiation can be low among recently isolated populations and at young migratory divides. We therefore advocate the use of high-throughput sequencing technology to generate the high-resolution genomic data necessary to detect regions of restricted gene flow extremely early in divergence. Association mapping for allopatric scenarios [61] and admixture mapping in hybrid zones [62] are both useful approaches to analyze the genomic architecture of migratory behavior [63]. Several candidate genes (e.g., *CLOCK*, *ADCYAP1*) appear influential in the expression of alternative migratory phenotypes [35,40,63]. Determining whether candidate loci show elevated patterns of genomic differentiation relative to background levels of divergence between individuals with alternative migratory phenotypes can provide additional insight into the role of migratory behavior in the accumulation of genetic divergence [64].

Step 3

Assess trait correlations (e.g., timing of migration, migratory route, phenotypic differences, habitat selection). As geographic isolation can yield divergence along numerous phenotypic axes, and many migratory divides have formed through secondary contact, it is important to identify and distinguish between traits that truly underlie patterns of mate choice and traits that are associated with the target of mate selection and therefore appear to influence patterns of genetic exchange. For allopatric scenarios, we advocate the use of variance partitioning to infer the role of migratory phenotype in genetic differentiation by examining the relative contributions of migration-related traits, traits unrelated to migratory behavior, and geographic distance to pairwise genetic divergence [65]. Multiple matrix regression analyses can be used to partition variation among correlated variables (e.g., spatial autocorrelation [66]).

Step 4

Evaluate the mechanistic basis of assortative mating and demonstrate its link to migratory behavior (only possible at migratory divides). We offer examples of correlational tests and experimental manipulations that can be used to tease apart the roles of multiple associated traits in assortative mating (Table 2). However, we recognize that experimental tests will only be feasible in a small number of study systems.

through diet at the site of tissue growth.
Trait: a measureable feature of a phenotype.

undertake inferior migratory routes, inherit maladaptive trait combinations, or experience suboptimal life history schedules associated with their migratory phenotype might limit gene flow through **post-zygotic reproductive isolation** [27,28]. For example, hybrids between populations that follow divergent migratory routes around a geographic barrier could experience increased mortality if they travel directly across the barrier during migration. Finally, post-zygotic selection against hybrids could lead to stronger prezygotic barriers via **reinforcement** [25]. In this scenario, selection for increased mate discrimination to avoid maladaptive hybridization between distinct migratory forms would yield greater divergence in traits associated with mate choice. Nonetheless, a comparative analysis of North American birds found that sister pairs that differ in migratory behavior exhibit lower rates of phenotypic divergence, suggesting that seasonal movements might be sufficient to maintain reproductive isolation even in the absence of phenotypic differentiation [29].
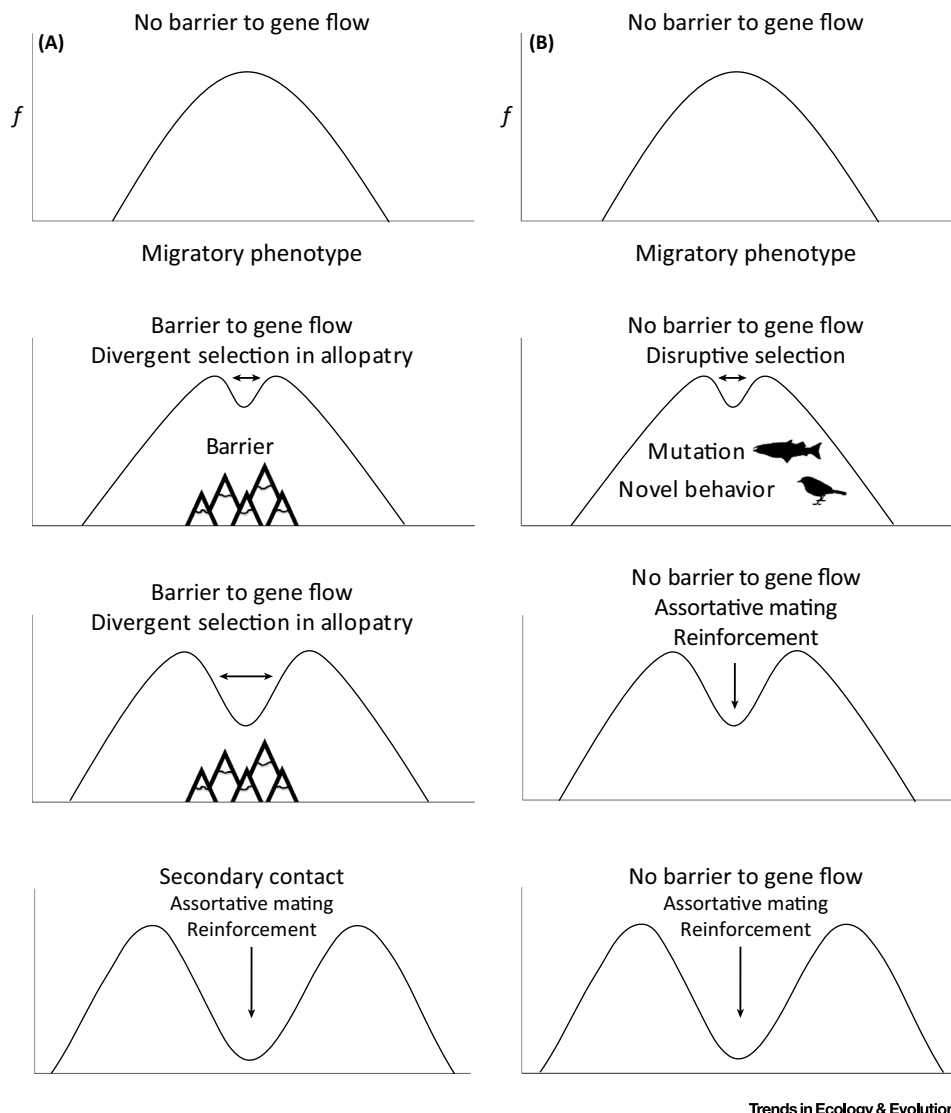
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Figure 1. A Diagram Outlining How (A) Secondary Contact and (B) Primary Divergence Could Lead to Reproductive Isolation by Migratory Phenotype and the Eventual Accumulation of Genetic Differentiation at Migratory Divides. Top panels represent panmictic populations with standing genetic variation in migratory phenotype. (A) Exposure to divergent ecological conditions and physical barriers to migration can lead to the evolution of divergent migratory strategies in geographically isolated populations. Depending on the duration of isolation in allopatry, alternative migratory forms may or may not accumulate significant genetic differences before secondary contact. When populations are brought into sympatry, existing trait differences might be sufficient to yield assortative mating by migratory phenotype. Otherwise, selection against hybrids could lead to reinforcement, further promoting trait divergence and the accumulation of genetic differentiation over time between individuals with alternative migratory phenotypes. (B) In primary divergence, a novel mutation affecting migration (e.g., salmon [42]) or a new migratory behavior (e.g., blackcaps [43]) can arise in a previously homogenous population. If individuals with different migratory phenotypes have equally high reproductive fitness, divergent migratory strategies will be maintained in sympatry. However, assortative mating and/or selection against hybrids, if present, will cause alternative migratory phenotypes to diverge from one another over time in traits involved in migration and eventually lead to the accumulation of genetic divergence between migratory forms.

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Divergent migratory phenotypes can also indirectly influence reproductive isolation through carry-over effects from the non-breeding season or differences in selection pressures between populations [30]. For example, exposure to divergent ecological conditions on the non-breeding grounds, such as the availability of dietary resources, the sensory environment in which signaling occurs, and the composition of predator and parasite communities, as a result of alternative migratory phenotypes could indirectly lead to divergence in traits involved in assortative mating, as sexual signals can rapidly diverge in response to ecological selection [31]. In addition, conflicting selection processes that arise as a byproduct of variation in migratory phenotype could promote divergence in signal traits and sexual preferences between populations [25]. For instance, comparative analyses of sedentary and migratory populations of several avian species have found that migratory males produce longer, female-directed songs while resident males produce shorter, repetitive songs effective in territorial interactions, which could potentially yield assortative mating on secondary contact [32,33].

A disproportionate amount of research on migratory divides has been conducted in avian systems, which are often characterized by strong breeding site fidelity, non-random mating, and variation in seasonal migration. Other taxonomic groups undergo migratory journeys that span multiple generations (e.g., insects [1]), choose mates, reproduce, and raise offspring in geographically distinct locations (e.g., bats [14]) and depend to various degrees on cultural transmission to undertake successful migratory journeys (e.g., wildebeest [1]). We currently lack sufficient information on migratory behavior to predict how divergent migratory phenotypes may influence patterns of genetic exchange in many systems with diverse life history strategies. However, the joint application of emerging genomic and tracking technologies in understudied systems will shed light on the prevalence of migratory divides and their broad importance to reproductive isolation and population divergence across animals as a whole.

Migratory Divides: Remaining Questions

While a variety of mechanisms are thought to underlie reductions in gene flow at migratory divides, the relative significance of possible isolating mechanisms, including pre- and post-zygotic barriers, in the evolution of reproductive isolation remains unclear. Recent advances in tracking techniques and high-throughput sequencing technology are making it possible to test these putative mechanisms for the first time by allowing researchers to measure variation in migratory behavior and link these movement patterns to gene flow and genomic ancestry in contact zones [5,8].

To advance our understanding of the evolutionary consequences of seasonal migration as new migratory divides continue to be discovered [20,34], we: (i) highlight remaining questions regarding the link between migratory phenotype and reproductive isolation by examining five case studies; (ii) propose a conceptual framework for studying the influence of divergent migratory phenotypes in the evolution of prezygotic isolation; and (iii) discuss how parallel developments in animal tracking and genomic sequencing technology can be leveraged to track patterns of gene flow as a function of migratory behavior and infer post-zygotic selection against hybrids at migratory divides.

Migratory Divide Case Studies

In Table 1 we summarize five case studies drawn from a variety of taxonomic groups to point out gaps in our understanding of how divergent migratory phenotypes contribute to reproductive isolation at migratory divides. In particular, few studies have investigated patterns of gene flow between individuals with divergent migratory phenotypes or matched observations of pairing data to paternity. Analyzing both within-pair and extra-pair mating could be revealing; for

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Table 1. Migratory Divide Case Studies[a]

System	Description	Biogeographic history	Axes of divergence	Maintenance of reproductive isolation	Gaps
Eurasian blackcap (*Sylvia atricapilla*)	Southwest (SW) migrants travel to the Iberian Peninsula and Africa; northwest (NW) migrants travel to the UK and Iceland from sympatric breeding grounds in Germany and Austria [43]	Primary divergence: novel NW direction evolved in the 1960s due to warmer winter conditions and supplemental feeding in the UK [43,38]	Genetic and phenotypic divergence (wing shape, beak width, and plumage coloration) [21]; timing of arrival on breeding grounds [26]	Prezygotic: assortative mating by timing of arrival [26], but SW migrants outnumber NW migrants on the breeding grounds and females arrive much later than males [38]; assortative mating by habitat selection on the breeding grounds [67] Post-zygotic: exhibit continuous wintering distribution from SW to NW Europe [36], but hybrids may orient in intermediate direction [39]	No study has examined patterns of extra-pair mate choice or selection against hybrids
Sockeye salmon (*Oncorhynchus nerka*)	Anadromous 'sockeye' migrate to the Pacific Ocean to mature; non-anadromous 'kokanee' remain in lakes until maturity; the two ecotypes often spawn in the same freshwater bodies [44]	Primary divergence: non-anadromous kokanee repeatedly evolved from anadromous sockeye [44]	Genetic and phenotypic divergence (body size); selection of oviposition sites [16]; ability to utilize carotenoids [45]	Prezygotic: assortative mating by body size [46]; habitat isolation due to disruptive selection on female oviposition sites [16]; sexual selection against green-colored hybrids [16] Post-zygotic: sockeye and kokanee produce viable, fertile offspring in hatcheries [45]; potential pleiotropic effects of carotenoid sequestration on **smoltification** in kokanee [16]	Few studies have evaluated the mechanisms maintaining reproductive isolation between distinct migratory forms; prezygotic mechanisms appear important but patterns of genetic exchange between ecotypes are unclear
Willow warbler (*Phylloscopus trochilus*)	*P. t. acredula* migrates southeast (SE) to southern Africa and *P. t. trochilus* migrates SW to west Africa from sympatric breeding grounds in Scandinavia [47]	Secondary contact following postglacial expansion from a common refuge population [24]	Little genetic differentiation at neutral loci [24] but divergence at two AFLP-derived markers; phenotypic divergence (body size, plumage coloration); timing of arrival [48]	Prezygotic: assortative mating proposed by timing of arrival [48]; no assortative mating between social mates by coloration, morphology, or isotopes [49]; hybridization is widespread [49] Post-zygotic: selection proposed against hybrids that take maladaptive migratory routes [49]	Post-zygotic selection against hybrids proposed as the only force that could explain the migratory divide but no study has examined extra-pair mate choice, hybrid fitness, or the migratory routes of hybrid individuals
Atlantic eels (*Anguilla rostrata* and *Anguilla anguilla*)	Larval *A. rostrata* and *A. anguilla* migrate on ocean currents from overlapping spawning grounds to streams and lakes in North America and Europe, respectively [50]	Secondary contact [50]	Genetic [51] and morphological divergence (vertebrae count) [50]; larval stage duration [51]; spawning time [52]	Prezygotic: assortative mating proposed through behavioral or ecological mechanisms (e.g., timing of spawning [52]) Post-zygotic: hybrids constitute a higher proportion of later life history stages (potentially due to increased hybrid survival) [50], but could experience lower fitness outside of hybrid zone; possibility of genetic incompatibilities	No studies have tracked patterns of genetic exchange on spawning grounds or hybrid fitness

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Table 1. (continued)

System	Description	Biogeographic history	Axes of divergence	Maintenance of reproductive isolation	Gaps
Swainson's thrush (*Catharus ustulatus*)	*C. u. ustulatus* migrates along the coast to southern Mexico and Central America, *C. u. swainsoni* migrates inland to overwinter from Panama to Argentina from sympatric breeding grounds in British Columbia [53]	Secondary contact following postglacial expansion from separate glacial refugia [23]	Genetic and phenotypic divergence (body size, plumage coloration, song); breeding habitat [23,54]; timing of arrival [55]; migratory orientation strongly associated with a genomic region of elevated differentiation [22]	Prezygotic: assortative mating proposed by timing of arrival, song, or habitat selection on breeding grounds [55] Post-zygotic: hybrids take intermediate migratory routes, but return rates are similar between hybrids and parentals [27]	No study has examined social or extra-pair mate choice or hybrid fitness at the divide; effect of intermediate migratory strategies for hybrid survival is unclear

[a]We examine five case studies of migratory divides to highlight the persisting gaps in our understanding of how divergent migratory phenotypes contribute to the generation and maintenance of prezygotic and post-zygotic reproductive isolation.

example, if rates of extra-pair paternity are high, and individuals mate assortatively by migratory phenotype with social but not extra-pair mates, studies that fail to track patterns of extra-pair mate choice could overestimate the importance of migratory behavior for reproductive isolation. In the following section, we present a framework to address these gaps by explicitly analyzing whether divergent migratory phenotypes lead to prezygotic isolation at migratory divides. While populations with divergent migratory strategies often differ in features that affect mating decisions, such as timing of breeding [26], phenotype [23], and habitat choice [67], comparatively little is known about the prevalence of maladaptive genotypes in hybrids, making assessment of post-zygotic barriers challenging.

A Conceptual Framework for Studying Pre-zygotic Isolation at Migratory Divides

Geographic Context and the Promotion and/or Maintenance of Reproductive Isolation

Many migratory divides in birds and mammals have formed through secondary contact between populations that evolved divergent migratory phenotypes in allopatry [17,23,24]; however, several divides, particularly in fish, are likely to have evolved through primary divergence (Table 1). We outline how secondary contact (Figure 1A) and primary divergence (Figure 1B) can lead to pre- and/or post-zygotic isolation by migratory phenotype and the eventual accumulation of genetic differences between populations. Regardless of the geographic context of divergence, the long-term expectation is largely the same if divergent migratory phenotypes contribute to prezygotic reproductive isolation at migratory divides. In divides formed through either primary divergence or secondary contact, traits involved in assortative mating by migratory phenotype will eventually exhibit a non-unimodal (e.g., bimodal) distribution, with little overlap between migratory forms, and individuals that pursue alternative migratory strategies will become genetically differentiated from one another. The rate at which genetic divergence accumulates will depend on the history of geographic isolation, the strength of assortative mating and selection against hybrids, the genetic architecture of migratory behavior, and the roles of social learning and phenotypic plasticity in generating variation in migratory phenotype. For recently evolved migratory divides, other measures, such as mate preferences, pairing decisions, and patterns of paternity, can be used to detect reproductive isolation in lieu of genetic divergence.

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The application of emerging technology to collect fine-scale data on variation in migratory routes, geographic location and habitat during the non-breeding season, and timing of arrival on the breeding grounds will facilitate the documentation of traits involved in prezygotic isolation. While many studies have used low-resolution genomic markers, such as microsatellites and allozymes, to detect regions of restricted gene flow between organisms with alternative migratory phenotypes [16,37], these markers lack the resolution necessary to detect genetic differentiation extremely early in divergence. We advocate the use of high-throughput sequencing technology to produce the high-resolution data necessary to examine the accumulation of genetic differentiation between closely related populations and identify the genomic regions underlying divergent migratory phenotypes [22].

The influence of cultural transmission and phenotypic plasticity on the accumulation of genetic differentiation at migratory divides is largely unknown. While both environmental and genetic drivers of alternative migratory phenotypes should eventually lead to genetic differentiation if individuals mate assortatively by migratory pattern, the effect of these processes on the rate at which genetic divergence accumulates is poorly understood. Cross-fostering experiments between individuals that differ in migratory behavior and the examination of correlations between genomic ancestry and migratory phenotype will allow researchers to investigate how mode of inheritance influences the strength of reproductive isolation and infer the relative contributions of genetics, learning, and phenotypic plasticity to the generation of alternative migratory strategies.

Testing the Mechanistic Basis of Assortative Mating Following Secondary Contact

Distinct migratory forms have often diverged in numerous traits in allopatry that could mediate assortative mating on secondary contact. Associations between traits due to divergence in allopatry can yield misleading conclusions about the contribution of seasonal migration to prezygotic isolation. To evaluate the role of migratory phenotype *per se* in the maintenance of reproductive isolation, we emphasize the need to compare allopatric and sympatric populations of the same species and comprehensively assess associations between diverse traits, such as timing of migration, propensity to migrate, morphological and phenotypic traits, habitat selection, and genomic ancestry (Box 1).

Once associations between behavioral and phenotypic traits have been identified, studies should attempt to tease apart these associations through statistical analyses and experimental tests. Observing changes in patterns of hybridization after an experimental manipulation is a particularly powerful way to detect a causal association between the manipulated trait and barriers to reproduction. This step will allow researchers to evaluate the mechanistic basis of assortative mating and its link to migratory behavior. In particular, sexually selected and naturally selected phenotypic traits [32,33], timing of arrival [26], habitat selection on the breeding grounds [67], and genomic ancestry could underlie assortative mating at migratory divides. In Table 2 we propose a series of correlational tests and experimental manipulations that will allow researchers to: (i) identify potentially important prezygotic isolating mechanisms that could maintain divergent migratory phenotypes at divides; and (ii) break apart trait correlations to infer the relative importance of these possible mechanisms of assortative mating. While certain systems are more amenable to experimental manipulation than others, correlational analyses alone will provide substantial insight into the traits underlying assortative mating at migratory divides. The application of this framework will shed light on the frequency with which migratory behavior directly (e.g., through assortative mating by timing of arrival) and indirectly (e.g., through carry-over effects from the non-breeding season) promotes reproductive isolation across a diversity of taxonomic groups.

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Table 2. Evaluating the Mechanistic Basis of Assortative Mating at Migratory Divides[a]

Basis of assortative mating	Prediction	Correlational test	Experimental manipulation
Phenotype – sexual selection	Mate selection maintains assortative mating (AM) by migratory behavior (e.g., signal traits predict migratory behavior)	Association between signal traits and migratory behavior, correlation between signal traits of mates	Remove or enhance signal differences and analyze patterns of genetic exchange (e.g., conduct mate choice trials with manipulated pheromone blends [56])
Phenotype – natural selection	Phenotype maintains AM by migratory behavior (e.g., wing length predicts migratory behavior)	Association between phenotypic traits and migratory behavior, correlation between phenotypic traits of mates	Remove or enhance trait differences and analyze patterns of genetic exchange (e.g., alter pigmentation patterns involved in background matching [57])
Behavior – timing	Timing differences maintain AM by migratory behavior	Association between timing of arrival and migratory behavior, correlation between migratory timing of mates	Remove timing differences and analyze patterns of genetic exchange (e.g., delay breeding of early-arriving males [58])
Behavior – habitat	Habitat selection maintains AM by migratory behavior	Correlation between wintering habitat and habitat selection on breeding grounds	Remove or enhance habitat differences and analyze patterns of genetic exchange (e.g., alter the stone cover on male territories [59])
Ancestry	Selection of genetically similar mates maintains AM by migratory behavior	Higher genetic relatedness between mates than expected by chance	Force heterotypic matings to expose post-zygotic isolating mechanisms (e.g., conduct hybrid crosses [60])

[a]Here we identify potential non-mutually exclusive mechanisms of assortative mating at migratory divides and consider how divergent migratory phenotypes can lead to prezygotic reproductive isolation within sympatric breeding populations. We propose correlational tests and, when feasible, follow-up experimental manipulations to infer the relative importance of possible mechanisms of assortative mating. The joint application of emerging tracking and genomic sequencing technologies will allow researchers to accurately assess and link various aspects of seasonal migration, including timing of arrival on the breeding grounds, non-breeding location (and potential carry-over effects from the non-breeding season), and genomic ancestry, to pairing decisions and patterns of paternity on the breeding grounds.

Detecting Post-zygotic Isolation Due to Divergent Migratory Phenotypes

Post-zygotic isolation can also restrict gene flow between groups that pursue alternative migratory strategies and might be particularly prevalent in certain systems, such as organisms that reproduce through broadcast spawning. To date, post-zygotic isolation as a function of migratory phenotype has been considered almost exclusively in songbirds, which, due to their small size, cannot support tracking devices that continuously transmit data in real time. As a result, researchers have attempted to infer post-zygotic barriers to reproduction by tracking the migratory routes of hybrid individuals using orientation funnels and geolocators, which must be retrieved to obtain tracking data [27,39]. In addition, studies have analyzed the return rates of hybrids relative to parentals [27] and compared the width of genetic and phenotypic clines associated with migratory divides with neutral expectations [23,24]. However, it is impossible to pinpoint the source of selection against hybrids and the role of seasonal migration *per se* in post-zygotic isolation using these approaches. In addition, documenting that hybrids follow intermediate migratory routes relative to parentals does not necessarily imply lower hybrid fitness. Finally, cultural inheritance of migratory route could limit the strength of post-zygotic barriers to reproduction in certain systems [1].

To investigate sources of post-zygotic isolation in more detail, we encourage research in non-avian systems, such as fish, caribou, and other large animals that can be tracked using acoustic

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telemetry or support GPS-based devices that transmit real-time data on animal positions. The high-resolution tracking data produced by these technologies will provide information on mortality rates, the avoidance of geographic barriers (e.g., deserts, mountain ranges) during terrestrial migrations, and the intermediate nature of migratory routes in hybrids, improving our ability to draw inferences about the strength of post-zygotic isolation due to divergent migratory phenotypes. Combined with detailed investigations into patterns of genetic exchange and hybrid fitness on the breeding grounds, fine-scale spatial data collected during the non-breeding season will provide insight into factors other than migratory route (e.g., carry-over effects from the non-breeding grounds) that promote selection against hybrids. In certain systems it may also be possible to conduct experimental crosses between individuals with alternative migratory phenotypes to examine inviability, sterility, and other sources of post-zygotic selection. Eventually, the further miniaturization of tracking devices, particularly those with GPS technology, and the development of satellite-based infrastructure [e.g., the ICARUS (International Cooperation for Animal Research Using Space) Initiative] to track the movements of small animals will open new opportunities to test the prevalence of post-zygotic isolation as a function of migratory phenotype in a wide range of taxonomic groups.

Concluding Remarks and Future Directions

Migratory divides represent a unique opportunity to improve our understanding of the role of seasonal migration in speciation; however, we lack a conceptual framework for demonstrating the role of divergent migratory phenotypes in prezygotic isolation. Here, we provide an overview of the numerous ways in which seasonal migration can contribute to genetic divergence among allopatric populations and reproductive isolation in areas of geographic overlap. We advance a conceptual framework to evaluate the relative significance of prezygotic isolating mechanisms that can underlie population differentiation due to variation in migratory behavior. In addition, we describe methods of inferring the contribution of post-zygotic barriers to reproductive isolation between individuals with divergent migratory phenotypes. Our framework discusses opportunities to leverage emerging technology in a wide range of taxonomic systems to track patterns of genetic exchange between individuals that pursue divergent migratory strategies and offers guidelines to explicitly examine the link between seasonal migration and the accumulation of reproductive isolating barriers. The generation of high-resolution spatial and genomic data advocated in this approach will not only provide direct insight into the role of migratory phenotype in the evolution and maintenance of reproductive isolation, but also inform other important aspects of seasonal migration, such as the repeatability of individual migratory routes, the genetic basis of migratory behavior [35,40], and the roles of phenotypic plasticity, social imprinting, and genetic variation in the generation of alternative migratory phenotypes (see Outstanding Questions). Growing evidence suggests that climate change can impact species distributions, influence the timing of migration, and select for the evolution of residence in certain migratory populations [41]. Understanding the lability of migratory behavior and the conditions under which seasonal migration promotes, maintains, or erodes barriers to genetic exchange in diverse taxonomic groups will become increasingly important as rapidly changing environmental conditions alter the journeys of migratory organisms in unpredictable ways.

Outstanding Questions

How often does migratory behavior directly versus indirectly promote reproductive isolation? Our framework provides guidelines for teasing apart potential mechanisms of prezygotic isolation and assessing their relative contributions to patterns of genetic exchange at migratory divides.

Have most migratory divides formed through secondary contact or primary divergence? The joint application of novel tracking and genomic methods to study migratory behavior and genetic ancestry across diverse taxonomic groups will allow generalizations to be drawn about the history of migratory divides.

Do populations with divergent migratory phenotypes exhibit stronger reproductive isolation than populations that possess similar evolutionary histories but lack divergent migratory phenotypes? The use of our framework to identify migratory divides and evaluate the strength of reproductive isolation will allow this question to be addressed through comparative methods.

How heritable are migratory phenotypes (routes, timing, orientation behavior)? Cross-fostering experiments and the use of high-throughput sequencing technology to identify genomic regions underling divergent migratory phenotypes will provide insight into the genetic basis of migratory behavior.

How repeatable are the migratory routes and non-breeding locations of individuals? The high-resolution spatial data gathered through the use of novel tracking devices will shed light on the repeatability of individual migratory phenotypes across years.

How often are migratory divides characterized by divergence in migratory timing alone? Emerging tracking technology will allow researchers to compare levels of divergence in timing of migration, migratory route, and non-breeding location at migratory divides for a wide array of taxonomic groups.

What biogeographic features predict the location of migratory divides? Further documentation of migratory divides will improve our ability to draw generalizations about their geographic location.

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