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Key words: Willow warbler, *Phylloscopus trochilus*, migration, tracking, behavioural genetics, light level geolocator

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In memory of my father Valdis Sokolovskis

Table of Contents

Abstract

Right before northern hemisphere's summer shifts to autumn small insect-eating birds start lengthy journeys to tropical regions. It is well established that temporary lack food and annual changes in weather are the ultimate reasons for this behavior. On a proximal level however, the process is poorly understood. Yet cross continental bird migration is a widespread phenomenon that has evolved several times independently. Different species execute specific migration routes with remarkable precision, over narrow species-specific time windows. Even more remarkable is that songbirds migrate alone thus relying only on internal cues. Routes and exact winter locations can vary substantially even between populations of one species. Crossbreeding experiments have proved that migration direction and timing are highly heritable. However, we are still clueless on which exact genes encode information necessary for migration. I studied the willow warbler *Phylloscopus trochilus*, a common songbird that breeds across the whole of northern Eurasia and spends the non-breeding period exclusively in tropical Africa. The far east Siberian subspecies *P.t. yakutensis* winters in south-east Africa and begin the journey by flying NW. Northern and Eastern European willow warblers *P.t. acredula* are migrating towards southern Africa and start the migration by heading SSE. Western European and southern Scandinavian populations *P.t. trochilus* head towards West Africa and initiate fall migration by flying SSW. European *trochilus* and *acredula* are nearly identical genetically except for two inversion polymorphisms on chromosomes 1 and 5, and presence or absence of a large repeat block (MARB-a). Far east Siberian *yakutensis* are genetically almost inseparable from Scandinavian *acredula,* except for a set of nearly fixed differences on a small region on chromosome 6. I deployed small tracking devices to record migratory routes of willow warblers from breeding sites in Sweden and eastern Russia and supplemented the tracking results with molecular methods to search for genes associated with the migratory behaviors.

Popular scientific summary

As the winter arrives to northern latitudes, weather becomes colder, days shorter, food more difficult to find. Brown bears enter dens and hibernate, emerald damselflies die and only eggs they had laid on underwater plants persist through winter, honey-bees huddle in their hives, eat the honey and shiver wings to warm themselves and each other. Small insect eating birds on the other hand fly to warm and tropical regions where food is abundant, and weather is nice. In spring when the nature wakes up from the slumber the bears crawl out of their dens, emerald damselfly eggs hatch and the new-born larvae begin their life, honeybees leave their hive and resume the busy buzzing and migratory birds announce their return by filling the landscape with their songs! One such bird is willow warbler.

Willow warblers are dull looking 10-gram songbirds with a beautiful song and an incredible skill to travel. Willow warblers from southern Sweden migrate to western Africa through Spain and western coast of the Sahara Desert. Willow warblers from northern Sweden cross Greece and migrate to southern Africa. It is quite a feat for such a small creature to safely navigate across two continents and back. But what makes these journeys even more fascinating is that these birds are born with genetic instructions that will dictate them, when and where to fly! Despite a lot of work, we still know very little about how and which genes control migratory behavior. To investigate this question, I used tiny tracking devices called geolocators to document migration routes of Swedish willow warblers and used molecular analysis to find out more about the genetic basis of long-distance migration. In central Sweden, the southern willow warblers that migrate southwest meet the northern willow warblers that migrate southeast. Southern and northern willow warblers look very similar, and their songs appear identical. They therefore very often pair up with one another and raise chicks together. In those cases, the chicks inherit some migration genes from the mother and some from the father. Research done at the Max-Plank institute in Germany on blackcaps (another small songbird) showed that if one parent has genes to migrate southwest and other parent has genes to migrate southeast, their chicks will fly in between and most likely perish in an attempt to cross the Mediterranean Sea and the Sahara Desert right in the middle! We tracked the migratory journeys of hybrid willow warblers from Jämtland and found two regions on the genome that very strongly associate with the migration direction. Even more interestingly we found that migration direction follows a dominant inheritance pattern. This means that unlike the blackcap hybrids, the hybrid willow warbler hybrids will either fly southwest or southeast. We still, however, do not know the exact genes involved and what they do, but with this work, we have made a significant step forward, so stay tuned because more exciting findings are on the way.

Populärvetenskaplig sammanfattning

När vintern kommer till de nordliga breddgraderna blir vädret kallare, dagarna kortare och det blir svårare att hitta mat. Brunbjörnar går in i sina iden, flicksländor dör och endast de ägg de lagt på undervattensväxter överlever vintern, honungsbin kryper ihop i sina kupor, äter honung och vibrerar med vingarna för att värma sig själva och varandra. Små insektsätande fåglar å andra sidan flyger till varma och tropiska områden där det finns gott om mat och där vädret är fint. På våren, när naturen vaknar ur sin slummer, kommer björnarna ut ur sina iden, flicksländornas ägg kläcks och larverna börjar sitt liv, honungsbina lämnar sin kupa och återupptar det hektiska surrandet, och flyttfåglarna tillkännager sin återkomst genom att fylla landskapet med sång! En av dessa fåglar är lövsångaren.

Lövsångaren är en oansenlig 10-gram tung sångfågel med en vacker sång och en otrolig färdighet att resa långt. Lövsångare från södra Sverige flyttar till västra Afrika via Spanien och längst västra kanten av Saharaöknen. Lövsångare från norra Sverige passerar istället över Grekland och flyttar till södra Afrika. Det är en stor bedrift för en så liten varelse att på ett säkert sätt navigera över två kontinenter och tillbaka. Men det som gör dessa resor ännu mer fascinerande är att dessa fåglar föds med genetiska instruktioner som bestämmer när och var de ska flyga! Trots mycket arbete vet vi fortfarande väldigt lite om hur och vilka gener som styr flyttningsbeteendet. För att undersöka den här frågan försåg jag över 450 lövsångare med små så kallade ljusloggrar, för att dokumentera deras flyttningsrutter. Därefter utförde jag genetiska analyser för att ta reda på mer om den genetiska grunden för dessa långväga flyttningar. I mellersta Sverige möter de sydliga lövsångarna som flyttar åt sydväst de nordliga artfränderna som flyttar åt sydost. Sydliga och nordliga lövsångare ser mycket lika ut och deras sång verkar identisk. De bildar därför mycket ofta par med varandra och föder upp ungar tillsammans. I dessa fall ärver ungarna en del flyttningsgener från modern och en del från fadern. Forskning som gjorts vid ett Max-Plank-institutet i Tyskland på svarthättor (en annan liten sångfågel) visade att om den ena föräldern har gener för att flytta sydväst och den andra föräldern har gener för att flytta sydost, kommer deras ungar att flyga i en riktning mellan dessa. Denna riktning skulle få hybriderna att korsa tvärs över Medelhavet och Saharaöknen, en resa sannolikt förknippad med förhöjd dödlighet. Vi följde resorna för lövsångarhybrider från Jämtland och hittade två regioner i arvsmassan som har ett mycket starkt samband med flyttningsriktningen. Ännu mer intressant är att vi fann att flyttningsriktningen följer ett dominant nedärvningsmönster. Detta innebär att till skillnad från svarthättorna kommer merparten av lövsångarhybriderna att antingen flytta sydväst eller sydost. Vi vet dock fortfarande inte exakt vilka gener som är inblandade och vad de gör, men med detta arbete har vi tagit ett stort steg framåt, så håll ögonen öppna, för fler spännande upptäckter är på väg!

Populārzinātnisks apraksts

Līdz ar ziemas tuvošanos Ziemeļu puslodē temperatūra pazeminās, dienas paliek īsākas un ēdiens paliek arvien grūtāk atrodams. Brūnie lāči ielien mājīgos midzeņos un snauž, rudens zaigspāres nomirst un tikai olas ko tās izdējušas uz zemūdens augiem pārziemo, medus bites sadrūzmējas stropos, un visu ziemu ēd vasarā sarūpēto medu. Mazi dziedātājputni kas pārtiek no kukaiņiem aizlido us tropiskām zemēm kur laikapstākļi ir jauki un kukaiņu ir pa pilnam. Pavasarī kad daba mostas, lāči izlien no midzeņiem, dīķu dibenos izšķiļas un dzīvi uzsāk zaigspāru kāpuri, bites izlido no stropiem un sāk meklēt ziedus ar nektāru, migrājošie dziedātājputni paziņo par savu atgriešanos peipildot mežus un pļavas ar savām dziesmām. Viens no šiem mazajiem putniņiem ir vītītis (*Phylloscopus trochilus*)!

Vītītis sver nieka 10 gramus, valkā pieticīgu brūngani pelēku ietērpu un dzied pasakaini skaistu dziesmu. Vītīši no Zviedrijas dienvidiem (pasuga *trochilus*) rudenī migrē uz Rietum Āfriku cauri Spānijai un Sahāras Rietumu krastam. Vītīši no Zviedrijas Ziemeļiem (pasuga *acredula*) migrē uz Dienvid un Austrum Āfriku cauri Grieķijai un Ēģiptei. Lieki uzsvērt ka priekš maza putniņa šie ceļojumi ir apbrīnas vērti, ņemot vērā ka mēs (visdrīzāk arī tu) bieži apmaldamies lasot sēnes puskilometru no mašīnas! Tālie putnu ceļojumi ir vēl jo vairāk apbrīnojami kad mēs aptveram ka šī uzvedība ir ģenētiski iedzimta. Katrs jaundzimis vītītis bez iepriekšējas pieredzes un vienatnē zina kad un uz kurieni ir laiks migrēt! Par spīti daudzu zinātnieku pūlēm mēs joprojām zinām ļoti maz par to kuri gēni un kā nosaka šo uzvedību. Lai pētītu šo jautājumu es izmantoju mazākās pieejamās ierīces dzīvnieku izsekošanai, ģeolokatorus. Kopā ar migrācijas maršrutiem mēs analizējām katra izsekotā vītīša DNS (Molekula kas satur teju visu infromāciju par to kas mēs katrs esam) lai atrastu gēnus kas nosaka uz kurieni katrs no viņiem grib migrēt. Zviedrijas vidienā abas pasugas *trochilus* un *acredula* satiekas un bieži sapārojas. Putnēniem kuru vecāki ir no dažādām pasugām piedzimst ar sajauktiem gēniem, daži kas liek migrēt Dienvid-Austrumu un daži kas liek migrēt Dienvidu-Rietumu virzienā. Leģendāri pētījumi Maksa Planka institūtā, Vācijā, ar melngalvas ķauķiem (vēl viens mazs dziedātājputns) ir parādījuši ka ja viens no vecākiem migrē uz Dienvid-Austrumiem un viens uz Dienvid-Rietumiem tad viņu bērni migrēs pa vidu un visdrīzāk nomirs centienos šķērsot Vidusjūru un Sahāru taisni pa vidu! Izmantojot ģeolokatorus un DNS analīzes mēs atradām divus DNS reģionus kas ļoti spēcīgi asociējas ar migrācijas uzvedību. Mūsu pētījums pārsteidzoši parādīja ka atšķirībā no melgalvas ķauķiem vītīšu hibrīdi ļoti reti lido pa vidu. Gandrīz visi hibrīdi migrēja vai nu uz Astrumim vai Rietumiem kā viens no viņu vecākiem. Mēs joprojām nezinām kuri gēni un kā tieši kontrolē šo uzvedību, taču ar šo pētījmu mēs esam veikuši ļoti nozīmīgu soli uz priekšu.

List of Papers

Paper I

Sokolovskis K., Lundberg M., Åkesson S., Willemoes M., Zhao T., Caballero-Lopez V., Bensch S. Migration direction in a songbird explained by two loci. (2023) Nature Communications. https://doi.org/10.1038/s41467-023-35788-7

Paper II

Sokolovskis K., Caballero- Lopez V., Åkesson S., Lundberg M., Willemoes M., Zhao T., Bensch S. Day time migration patterns in willow warblers differ between the western and eastern flyway. Submitted.

Paper III

Sokolovskis K., Caballero- Lopez V., Åkesson S., Lundberg M., Willemoes M., Zhao T., Bensch S., Loop migration patterns in Swedish Willow Warblers. Manuscript.

Paper IV

Sokolovskis K., Lundberg M., Liedvogel M., Åkesson S., Solovyeva D., Willemoes M., Bensch S., Phenotypic and genetic characterization of the East Siberian Willow Warbler (*Phylloscopus trochilus yakutensis* Ticehurst, 1935) in relation to the European subspecies. (2019) Journal of Ornithology. doi.org/10.1007/s10336-019-01653-y

Paper V

Sokolovskis K., Bianco G., Willemoes,M., Solovyeva D., Bensch S. & Åkesson S. Ten grams and 13 , 000 km on the wing – route choice in willow warblers *Phylloscopus trochilus yakutensis* migrating from Far East Russia to East Africa. (2018) Movement Ecology. doi.org/10.1186/s40462-018-0138-0

Paper VI

Lundberg M.*, Sokolovskis* K, Zhao T., Red'kin Y., Bensch S. Genomic divergence between Scandinavian and East Siberian willow warblers *Phylloscopus trochilus*. Manuscript. *Shared first authorship.

Author's contribution to the papers

Paper I

Conceptualization: S.B., K.S. and S.Å. Tracking data analysis: K.S. and M.W. Laboratory work: V.C.L., T.Z., and K.S. Field data collection: K.S., M.Lu., S. Å., M.W., T.Z., V.C.L., and S.B. Writing—original draft: K.S. with input from S.B. Writing—review and editing: K.S., M.Lu., S.Å., M.W., T.Z., V.C.L., and S.B.

Paper II

Conceptualization: S.B., K.S. Tracking data analysis: K.S. Field data collection: K.S., M.Lu., S.Å., M.W., T.Z., V.C.L., and S.B. Writing – first draft: K.S. Writing - review and editing: K.S., M.Lu., S.Å., M.W., T.Z., V.C.L., and S.B.

Paper III

Conceptualization: S.B., K.S. Tracking data analysis: K.S. Laboratory work: V.C.L., T.Z., and K.S. Field data collection: K.S., M.Lu., S.Å., M.W., T.Z., V.C.L., and S.B. Writing – first draft: K.S. Writing - review and editing: K.S., M.Lu., S.Å., M.W., T.Z., V.C.L., and S.B.

Paper IV

Conceptualization: S.B., K.S. Field data collection: K.S., D.S. Laboratory work: K. S. Writing – first draft: K.S. Writing - review and editing: K.S., M.Lu., M.Li., S.Å., D.S., M.W., S.B.

Paper V

Conceptualization: S.B., S.Å. Tracking data analysis: M.W., G.B. Field data collection: K.S., D.S., Writing – first draft: S.Å. Writing - review and editing: K.S., M.Lu., S.Å., M.W., S.B.

Paper VI

Conceptualization: K.S., S.B., M.Lu. Museum skin sampling: K.S., Y.R. Laboratory work: K.S. Genomic analysis: M.Lu. Writing - first draft: S.B., K.S., M.L. Writing - review and editing: S. B., K.S., M.Lu., T.Z., Y.R., S.B.

Kristaps Sokolovskis (K.S.), Staffan Bensch (S.B.), Susanne Åkesson (S.Å.), Mikkel Willemoes (M.W.), Violeta Caballero-López (V.C.L.), Tianhao Zhao (T.Z.), Max Lundberg (M.Lu.), Miriam Liedvogel (Mi.Li.), Diana Solovyeva (D.S.), Giuseppe Bianco (G.B.), Yaroslav Red'kin (Y.R.).

Abbreviations

|	
InvP-Ch1	Inversion Polymorphism on Chromosome 1
InvP-Ch5	Inversion Polymorphism on Chromosome 5
MARB-a	Migration Associated Repeat Block in *acredula*
Mb	Mega base pair
Kb	Kilo base pair
SNP	Single Nucleotide Polymorphism
FDF	Full Day Flight
MCL	Mediterranean Crossing Longitude

Introduction to genetics of bird migration

As early as in 1702 Von Pernau noted that a migratory bird "… is driven in due time by some secret impulse and obeys it." (In Stresemann 1947). At the end of northern summer this "secret impulse" pushes billions of individual birds from the northern hemisphere to take the wing to reach very specific winter sites in the tropics only to return to the breeding territories when the winter has passed (Hahn et al., 2009). Similar seasonal movements occur across the animal kingdom: insects (Gao et al., 2020), crustaceans (Nieves-Rivera & Williams, 2003), fish (Albert et al., 2006), mammals (Wilkinson & Fleming, 1996) and reptiles (Papi et al., 2000). Lengthy migration has very likely been a commonly adopted survival strategy already in pre-avian forms of early Cretaceous dinosaurs (Suarez et al., 2017) and might even trace back to the very dawn of multicellular life (Hou et al., 2008). The "ultimate" causes for why migration has evolved are reasonably well understood and boil down to the avoidance of temporarily unfavorable conditions and exploitation of seasonal resource peaks (Alerstam et al., 2003; Winger et al., 2018). However, the "proximate" mechanisms of how an individual bird arrives to a decision on which direction to fly is an unsolved puzzle (Liedvogel et al., 2011). An undisputable conclusion however is that long distance bird migration has a strong genetic basis, which has been confirmed with experiments in the wild (Perdeck, 1958), common garden studies (Berthold, 1991) and observations in nature (Willemoes et al., 2014). We know that the photoperiod in combination with an internal genetically controlled clock tells the bird when to start the journey (Gwinner, 1972). We also know that the magnetic sense (Gwinner & Wiltschko, 1978) and stellar compass (Emlen, 1975) provide sophisticated tools for orientation. But aside from the fact that the flight direction and migration distance are genetically inherited (Berthold, 2003), we know virtually nothing about how and which genes that let the birds to know which direction to take.

The migration routes of birds are often counter-intuitive and much more elaborate than common sense would suggest (Sutherland, 1998; Ruegg & Smith, 2002). Long-lived social migrants are much better at adjusting their migratory routes as a response to a changing environment than short-lived insectivores that typically migrate in solitude (Sutherland, 1998). White storks *Ciconia Ciconia* from western Europe and eastern Europe start autumn migration flying southwest and southeast,

respectively. However, if a young white stork with the genetic drive to migrate southwest joins a flock that is lead southeast by experienced adults, the juvenile will ignore the innate drive and follow the group (Chernetsov et al., 2004). The role of cultural transmission of the migratory behavior was highlighted in a recent experiment where juvenile hand-reared black-tailed godwits *Limosa limosa* were displaced from the Netherlands to Poland. The Dutch godwits released in Poland migrated very similar to the local Polish godwits (Verhoeven & Loonstra, 2020) and even returned to breed in Poland. In long distance songbird migrants (for example Sylvoidea warblers), migration is a strictly solitary activity. Moreover, the details of the migratory program (timing, flight direction, fattening strategy, stopover location, winter range) are typically specific for the species, and at times, even for populations of the same species (Liedvogel et al., 2011). For example, the five closely related *Phylloscopus* species, willow warbler, greenish warbler *P. trochiloides*, wood warbler *P. sibilatrix* and arctic warbler *P. borealis* breed sympatrically in the European part of Russia (Karelia). All five are obligatory long-distance migrants. All five are ecologically nearly identical and can nest in the same hectare of land, and yet a willow warbler from Karelia will migrate to southern Africa, wood warbler to western Africa, arctic warbler to Philippines and the greenish warbler to India (Shirihai & Svensson 2018, **Figure 1**). Species like the above mentioned *Phylloscopus* showcase large and rather dynamic breeding areas. In contrast, winter grounds across the migratory bird species appear to be rather inert to change. It is a common pattern where birds of one species from a large breeding range all funnel to much smaller wintering area. Hence, it seems that there is a strong signature of a historical legacy determining the migratory behavior today.
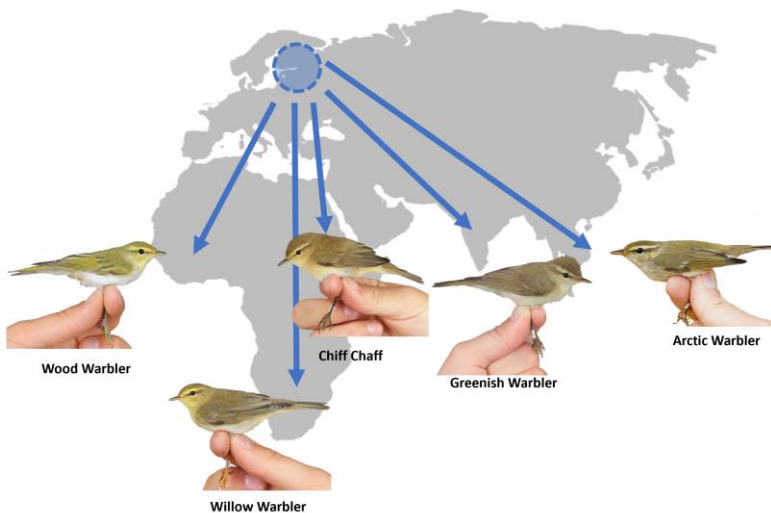
Figure 1. Diverse wintering grounds of five sympatric leaf warbler species. Photos of chiffchaff, wood, willow and greenish warblers kindly provided by Ottenby Bird Observatory and photo of the arctic warbler by Gabriel Norevik.

Migration evolves fast

Migration seems very labile; it can be gained or lost over short evolutionary timescales (Dufour et al. 2020, **Figure 2**). Examples of rapid evolution of migration are not uncommon even in the very narrow span of the recorded history (Sutherland 1998). In the 16th century, the serin *Serinus serinus* for example occurred only around Mediterranean region and was a strictly resident species. By early 20th century it had expanded all the way to Scandinavia and at these latitudes became an obligatory migrant (Kinzelbach, 2004; Mayr, 1926). Migration does require many coadaptations but most if not, all phenotypic characteristics enabling long distance migration are probably modifications of already pre-existing characteristics most birds possess (like the famous analogy with "Spandrels of San Marco" introduced by Gould & Lewontin (1979)). For instance, the ability to accumulate fat for the migratory journeys in sedge warblers *Acrocephalus schoenobaenus* likely employs the same physiological machinery that the sedentary marsh tits *Poecile palustris* use to accumulate fat over the day to survive a cold winter night (Rappole, 2013). It is thus plausible to assume that a few key changes in the gene sequence or expression pattern can have cascading effect on the entire migratory phenotype.
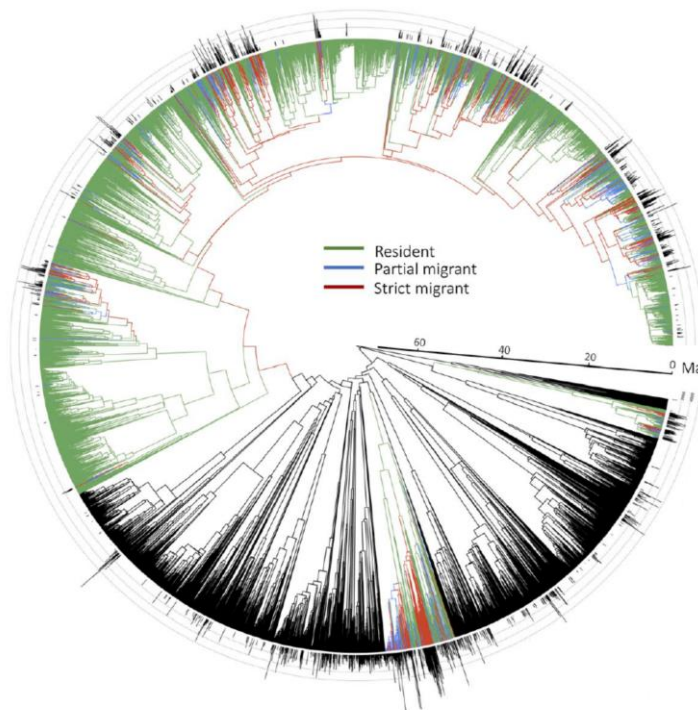
Figure 2. Bird phylogeny with reconstructed character states for the migratory phenotype. Figure modified from Dufour *et al.* (2020).

Breeding philopatry as the driving force

Several extensive reviews on evolution of bird migration have already been written e.g., Berthold (1999), Alerstam *et al.* (2003), Newton (2008), Rappole (2013). Two major and much debated hypotheses on the origin of bird migration that I want to bring up are, the "southern home" (birds that breed in tropics expanded to northern hemisphere to breed and become migratory in higher latitudes) and the "northern home" (birds being sedentary in high latitudes begin to migrate south for wintering). For example, the observation that migratory Holarctic species usually have close relatives in the tropics that are sedentary or partially migratory, has been interpreted as support for the" southern home" hypothesis (Winger et al., 2012). On the other hand, careful phylogenetic analyses of character states have in some cases supported the "northern home" hypothesis (Winger et al., 2014). The previously mentioned debate however may have exhausted its usefulness. Winger *et al.* (2018) suggests that at the conceptual level it is the breeding site philopatry and the location of suitable winter areas that shape current migratory routes. Because of spatial climate patterns along latitudes, most migrations happen to take place along a north-south axis. There are however some notable exceptions to these north-south migration itineraries. Ancient murrelets *Synthliboramphus antiquus* for example, migrate from Canada to Japan and back, from west to east with almost no change in latitude (Gaston et al., 2015). An example from passerines is the black-headed bunting *Emberiza melanocephala* that migrates from southern Italy and the Balkans to India with very little change in latitude but a massive longitudinal shift (Ćiković et al., 2021). Yet another species illustrating that migration is merely an adaptation to overcome temporary periods with adverse conditions is the common poorwill *Phalaenoptilus nuttallii*, that in fact truly hibernates (Jaeger, 1948). The above-mentioned examples emphasize that migration plays the same role as hibernation in mammals or diapause in insects (Winger et al., 2018). The line of thought that there is a strong tendency to maintain the same breeding site, is also supported by the fact that despite the often-occurring migratory connectivity (Webster et al., 2002), a precise breeding site fidelity is generally much stronger than winter site fidelity (Newton, 2008). At first this seems to be contradictory with the fact that the breeding ranges are dynamically changing, and winter ranges are generally inert to change (Bensch, 1999; Böhning-Gaese et al., 1998). However, Winger's theory still stands if we realize the distinction between natal and breeding dispersal. Adult individuals, that already have lived through one breeding season will often return the next year to breed in the same territory (a phenomenon known as breeding philopatry). On the other hand, natal dispersal, the distance from the place where a bird hatched to the place where the bird settled for its first breeding attempt, generally operates on a significantly larger geographical scale than breeding dispersal (Newton, 2008; Paradis et al., 1998). Natal dispersal on a population level is a mechanism that allows the breeding ranges to change even in the face of limited breeding dispersal.

Hypothetical orientation patterns and inheritance modes

There are two main hypotheses proposed for what type of inherited information might be contained in the genetic migration program that enable naïve birds to reach their wintering grounds (**Figure 3**).

"Clock and Compass": separate genes for direction, timing and duration of the journey.

Albert C. Perdeck in the mid-20th century ringed and displaced >11 000 common starlings *Sturnus vulgaris* from the Netherlands to Switzerland. Ringing recoveries of these birds suggested that due to prior experience, adults can compensate for the displacement and reorient toward their real destination area. Inexperienced juveniles after displacement however continued to fly in the set direction and for the set amount of time as if they were not displaced. This juvenile behavior was the origin of the term "Clock and Compass" (Perdeck, 1958, 1967). In orientation studies it has even been noted that garden warblers *Sylvia borin* studied in orientation cages change the migration direction according to what they should have done in wild (Gwinner & Wiltschko, 1978). Garden warblers in Eberhard Gwinner's and Wolfgang Wiltschko's experiment started with orienting south-west and later reoriented south. This pattern matches their natural flight south-west to the Iberian Peninsula followed by a southward flight along the western edge of Africa. This is what would be expected under "Clock and Compass" orientation. Thorup and colleagues caught white crowned sparrows *Zonotrichia leucophrys* on the western coast of US during fall migration and displaced them to the eastern coast. The researchers fitted these birds with radio transmitters and managed to track their movements for >100 km from the release site. The results were clearly supporting the "Clock and Compass" hypothesis: adults compensated for the displacement whereas juveniles did not (Thorup et al., 2007).

"Goal-area Navigation": set of genes determining the destination and/or staging areas regardless of the starting position.

Jørgen Rabøl (1978), after analyzing ring recoveries of three European passerine species, suggested that it is in fact the geographic location of the wintering area (or the stopover sites) that is inherited, rather than the direction or timing.

The "Clock and Compass" orientation is relatively simple to comprehend but is not fully compatible with the fact that the migratory routes of many species must have changed dramatically during Holocene. After the last glaciation, when the majority of migrant species had been confined to small refuge areas, the ice sheets contracted, and the breeding ranges expanded dramatically. As the breeding ranges expanded and winter grounds remained the same, the populations at the edges of the new breeding grounds continuously needed to adjust the autumn migration direction in order to reach the ancestral wintering grounds. If these directional changes are genetically determined, as proposed by the "Clock and Compass" model, the

required rate of changes in coding sequences might be too high for novel adaptive mutations to occur. The "Goal-area navigation" is however much more difficult to comprehend. But in a thought experiment where we assume that the "Goal-area navigation" is true, previously problematic observations seem logical. For example, the common rosefinches *Carpodacus erythrinus* reached Scandinavia from Central Siberia about one hundred years ago and still winter in the same area of southern Asia (Pavlova et al., 2005). Rosefinches that expanded westwards had to change migration direction and timing to optimally reach the old wintering site. Under the "Clock and Compass" scenario, the new flight direction and timing would either require *de novo* genetic mutations or selection on standing genetic variation. Large amount of standing genetic variation in the form of recessive alleles affecting migratory direction might be possible if migration direction is a quantitative trait determined by many genes with small effects, which so far seems not be the case (Berthold, 1999; Sokolovskis et al., 2023). Mutations are rare, vast majority of them are neutral and only a small percentage are either detrimental or beneficial (Kimura, 1968). It therefore would imply that the common rosefinches and other migratory songbirds that have quickly expanded their breeding ranges would have obtained the necessary mutations to optimally changing migration direction and timing as the colonization of the continent continued. Aside from this verbal argumentation, "Goal area navigation" has gained empirical support (Åkesson et al., 2005; Thorup et al., 2011) and thus deserves serious consideration.
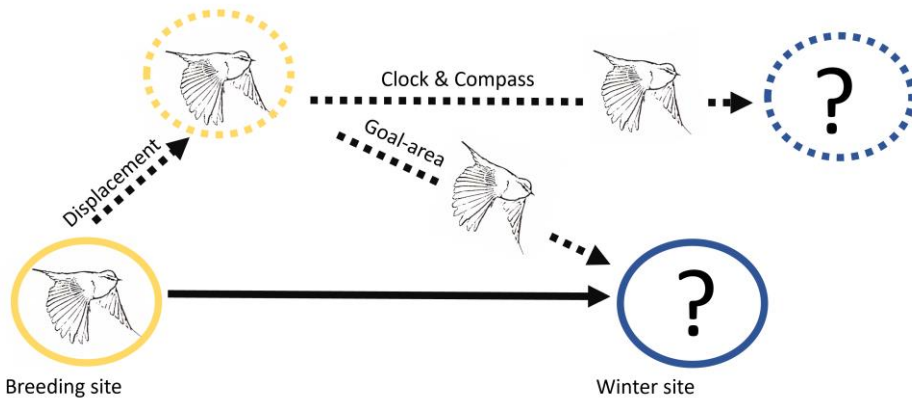
Figure 3. Schematic depiction of an ideal displacement experiment and possible outcomes under "Clock and Compass" and "Goal-area navigation" hypothesis.

To elucidate whether it is "Goal-area navigation", "Clock and Compass" orientation or a combination of both, it is necessary to replicate Perdeck's experiment in true solitary migrants. Starlings migrate in social flocks. The displaced juveniles might have joined flocks of local birds at the release site (Piersma et al., 2020). To do this,

we must deploy transmitters that would provide daily locations in real time and displace eggs before they hatch in order to eliminate maternal effects on development of the individual's migratory program. The presently available geolocators and GPS tags that are suitable for use on small birds only provide information of the surviving birds that return to the tagging site making it impossible to study how selection is operating on the variation of migration direction in populations. At this moment however, no GPS transmitters are small enough to be deployed on *phylloscopus*-sized passerines.

Components of migratory program likely under genetic control

Decades of experiments using the so called "Emlen funnels" and magnetic coils have helped to elucidate several components of the innate migration program that are very likely to be inherited. Below I summarize some of the most important published results to date.

Timing, when to start the migration

Dates of the start of migration are consistently different between species and to a large degree depend on an internally controlled clock (Åkesson et al., 2017). Early on, it was shown that chiffchaffs and willow warblers in captivity, sheltered from any environmental stimuli, initiate the migratory restlessness in appropriate species-specific times (Gwinner, 1972). Captive blackcaps kept in constant conditions (temperature, light and food availability) begun to express migratory restlessness at the same time as the wild populations they had been taken from leave the breeding grounds (Berthold & Helbig, 1992). From several of the candidate genes suggested to affect migration timing the *Clock* gene has received the most attention (Bazzi et al., 2016). However, a careful inspection, also including published negative results, clearly shows that its role may have been overestimated. Variation on the *Clock* gene does not have a large effect (Parody-Merino et al., 2019). Genomic analysis of migration timing in American kestrels *Falco sparverius* suggest that the timing is a polygenic trait and regulatory elements play a key role, whereas core genes involved in circadian activity patterns (e.g., *Clock*) remain conserved (Bossu et al., 2022).

Duration of how long to travel

Elaborate experiments carried out at the Max Planck institute of Ornithology scored the migratory activity ("z*ugunruhe*") in captivity of sedentary and migratory blackcaps (Berthold et al., 2003). Afterwards, the F1 hybrids were tested for "*zugunruhe*" in the exact same manner, and it was found that 40% of the birds displayed typical migratory activity. In addition, the duration of the hybrid "*zugunruhe*" was about half of that of the fully migratory blackcaps. A similar although less conclusive result was found when testing F1 generation hybrids of short distance migrating black redstarts *Phoenicurus orchruros* and long-distance

migrating common redstarts *Phoenicurus phoenicurus*. Overall, it does seem that the duration of migratory activity (proxy for the total migration time) is a quantitative multi-locus trait (Berthold, 2003).

Autumn migration direction

Blackcaps from southwest and southeast migratory populations were crossbred and F1 generation hybrids were shown to orient on average, intermediately to the parents (Helbig, 1991). This study is to date the best empirical demonstration of the genetic control of direction of bird migration. In addition, this team of researchers successfully crossbred the F1 hybrids. The preferred autumn migration direction of F2 hybrids showed larger variation than the F1 hybrids and segregated in more discrete clusters. This is in line with a Mendelian inheritance pattern of a trait that is controlled by a small number of genes with large effects (Helbig, 1996). A single most promising candidate gene for migration direction so far is *VPS13A* gene on Z chromosome that was found studying golden-winged *Vermivora chrysoptera* and blue-winged warblers *V. cyanoptera* (Toews et al., 2019).

Spring migration direction

To my knowledge, there is only one published experiment that provides evidence for the spring migration being innate and not a simple result of path integration mechanism. Captive garden warblers *Sylvia borin* that after being held captive throughout the winter, in spring exhibited typical "*zugunruhe*" and oriented consistently along a normal migratory direction, even including changes in direction (Gwinner & Wiltschko, 1980).

Affinity for specific parameters of the Earth's magnetic field

It is widely accepted that birds possess a magnetic sense, even though the mechanistic details are still debated, and no direct proof of specific receptors exists (Mouritsen, 2018). Experiments have shown that birds seem to respond to several properties of earth's magnetic field: declination (Chernetsov et al., 2017), inclination (Åkesson *et al.* 2001) and intensity (Dennis et al., 2007). Hypothetical mechanisms to perceive magnetic field components are cryptochromes in the retina that may enable birds to "see" the magnetic field (Pinzon-Rodriguez et al., 2018), magnetites near the trigeminal nerve (Kishkinev et al., 2013; Kirschvink & Gould, 1981) or symbiotic magneto-tactile bacteria (Natan & Vortman, 2017). The types of cryptochromes and specifics of magnetite molecule aggregations could in principle be directly encoded genetically and through a positive reward mechanism determine preferred migration direction. Symbiotic bacteria on the other hand would most likely be transmitted either from the environment or the mother through the egg laying. There may still be genetic components that make the host more welcoming to certain strains of bacteria.

In an elegant experiment, Fransson *et al.* (2001) caught juvenile thrush nightingales *Luscinia luscinia* in Sweden during autumn migration and kept them in aviaries surrounded with magnetic coils that enabled manipulation of the magnetic field intensity and inclination. The experimental group was kept under a magnetic field mimicking that of Egypt. These birds accumulated a lot more fat, presumably to prepare for the Sahara crossing, than birds that were placed in cages with a magnetic field corresponding to the actual location in Sweden. While Fransson *et al.* (2001) measured the response in fat accumulation in autumn, Kishkinev *et al.* (2015) captured reed warblers *Acrocephalus scirpaceus* during active spring migration in Kaliningrad and virtually displaced the birds by keeping them in magnetic field condition of a place 1000 km to the east and measured their preferred direction in "Emlen funnels". The virtually displaced reed warblers compensated for displacement and reoriented accordingly. It is difficult to analyze the role of each component of the magnetic field separately as they are not uniformly distributed across the globe and often covary (Boström et al., 2012). Nevertheless, recent work on Manx shearwaters *Puffinus puffinus* (Wynn et al., 2020) and common reed warblers (Wynn et al., 2022) suggests that migratory birds have a preference for a specific magnetic inclination value and use it as a stop sign when returning to the breeding site in spring. Whether it is genetic or learned is still not known. Alerstam & Högstedt (1983) reported results suggesting that ability to orient according to magnetic field parameters in pied flycatchers *Ficedula hypoleuca* is being calibrated already in the nestling stage. To conclude, it is within the realm of possibilities that there is an innate preference for wintering at a specific combination of magnetic field parameters. This however has not been tested yet.

My thesis

Study system: the willow warbler *Phylloscopus trochilus*

The willow warbler is a small 10-gram, old world passerine that breeds in northern Eurasia from the coast of Pacific to the coast of Atlantic. It is an abundant species with a population size between 400 and 650 million breeding individuals (birdlife.org). Birds breeding in southwestern Europe (ssp. *P. t. trochilus* Linnaeus, 1758) are the smallest and characterized by having a lot of yellow in the plumage. In autumn, they migrate towards southwest to wintering grounds in western Africa. Northern and eastern Europe is populated by longer winged birds (ssp *P. t. acredula*, Linnaeus, 1758) that have on average less yellow color in the plumage (**Figure 4**). They migrate towards south/southeast to winter quarters in southern and eastern Africa. The easternmost populations from northeast Siberia (ssp. *P.t. yakutensis* Ticehurst, 1935) are even longer winged and have hardly any yellow in the plumage. The subspecies *yakutensis* begins the autumn migration by heading northwest (Sokolovskis et al., 2018, 2019). First to suggest that *P. t. trochilus* and *P. t. acredula*, the two Scandinavian subspecies, migrate differentially was the Danish ornithologist Finn Salomonsen (Salomonsen, 1928, 1955). Salomonsen's inferences hinged on indirect data of differences in morphology and timing of passage. In 1980s Hedenström & Pettersson (1987) took advantage of the accumulated recoveries of willow warblers ringed in Fennoscandia and showed that indeed *trochilus* and *acredula* migrate differentially. Later research making use of analyses of stable isotopes from winter grown remiges identified that between latitudes 62 and 64 °E Scandinavian willow warblers may winter anywhere in Africa (Bensch et al., 2009; Chamberlain et al., 2000). The two subspecies *trochilus* and *acredula* meet in a ~250 km wide migratory divides in central Scandinavia and in eastern Europe (Bensch *et al.* 2009, Lundberg *et al.* 2017) where they breed almost panmictically (Liedvogel et al., 2014). Lerche-Jørgensen *et al.* (2017) conducted the first tracking study on willow warblers and presented direct evidence that at least the Danish *trochilus* migrate as Salomonsen predicted. Lundberg *et al.* (2017) compared genomes of *acredula* and *trochilus* and identified three inversion polymorphisms setting the subspecies apart, located on chromosome 1 (11 Mb, 146 coding genes), chromosome 3 (13 Mb, 135 coding genes) and on chromosome 5 (4 Mb, 53 coding genes). All these three regions have been confirmed to be inversion polymorphisms (Lundberg et al., 2023), meaning that recombination between the

two different haplotypes virtually does not happen and therefore, two separate sets of adaptations can evolve even in sympatry. The region on chromosome 3 has been shown to be related to the altitude and climate of the breeding site, not to the migratory behavior (Larson et al., 2014), and is thus not investigated in my thesis work. From here on we will refer to the inversion polymorphisms on chromosomes 1 and 5 as InvP-Ch1 and InvP-Ch5 respectively. One more marker circumstantially associated with the migratory behavior in willow warbler was first identified based on an AFLP protocol but is now genotyped with a qPCR protocol to detect the copy number of a transposable element specific to *acredula* (Bensch et al., 2002; Caballero-López et al., 2022). This marker as presence or absence of a > 12 Mb long repeat block that we will further call MARB-a (Migration Associated Repeat Block in *acredula*). From all the candidate regions in our study system, MARB-a is the least understood (we do not know its location on the genome, nor any functional genes it may carry). Ironically, MARB-a is also the marker with the strongest association to migratory direction in willow warblers (Sokolovskis et al., 2023).

Figure 4. Two willow warblers from the Swedish migratory divide. The individual to the left presents a typical grey-brown *acredula* plumage and the individual to the right a typical yellow-greenish *trochilus* plumage. Many birds are intermediates and both extremes of the plumage can be found anywhere in Sweden precluding use of coloration in identifying genetic origin. Photo: Harald Ris.

Core methods widely used throughout this thesis

Phenotyping

We tagged adult male willow warblers with light level geolocators produced by Migrate Technology Ltd (Intigeo-W30Z11-DIP 12 × 5 × 4 mm, 0.32 g, **Figure 5**). Loggers were attached to birds using a nylon string, originally meant for repairing mist nets, in a "leg loop" harness (Naef-Daenzer, 2007). The geolocators we used recorded maximum light intensity every five minutes and archived this data. Upon recapture of a tagged bird in the following spring the logger was retrieved. Data on light intensity with precise time stamps could then be converted to latitude and longitude estimates which we used to describe migratory routes.

Genotyping

Genotyping of the inversion polymorphisms on InvP-Ch1 and InvP-Ch5 was done using a qPCR SNP assays for one informative SNP per inversion (originally developed by Zhao *et al.* 2020). Probes and primers were produced by Thermo Fisher Scientific and were designed using the online Custom TaqMan® Assay Design tool. We used BioRad CFX96™ Real-time PCR system (Bio-Rad Laboratories, CA, USA). The qPCR method that we used to assess the presence of MARB-a was based on an assay that quantifies the copy number of a novel TE that has expanded in *acredula* (Caballero-López et al., 2022). Allopatric *trochilus* have from 0 to 6 copies whereas allopatric *acredula* have from 8 to 45 copies. Birds with >7 TE copies were considered to have one or two alleles of MARB-a.
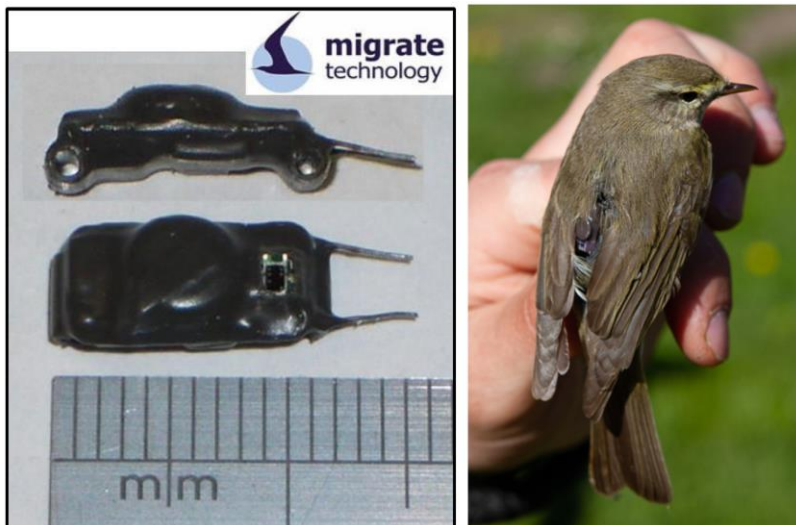
Figure 5. To the left a geolocator (from Fox, 2021). To the right a tagged willow warbler with the geolocator visible. Photo: Harald Ris.

Contributions to the research field from this thesis

Inheritance mode of the migratory phenotype (Paper I)

Migration direction

We deployed a total of 466 geolocators on willow warblers breeding across Sweden and retrieved data from 72, of which 51 were from the migratory divide. The primary goal of this effort was to identify which of the three candidate loci (InvP-Ch1, InvP-Ch5 and presence or absence of MARB-a) associate with differences in the migratory behavior. The results strongly suggest that migration direction in willow warblers follows a dominant inheritance pattern. The InvP-Ch1 carries an allele with a dominant effect for SW migration direction and MARB-a locus carries or is associated to an allele with a dominant effect for SE migratory direction. In addition to this, we also find that MARB-a epistatically suppresses effects of InvP-Ch1. In fact, presence or absence of MARB-a alone explains 64% of variation in autumn migration direction (**Figure 6**). This is in line with the classical prediction that migration direction is determined by a small number of large effect genes (Berthold 2003) but does not support a codominant inheritance mode of migration direction (Berthold 2003, Delmore et al. 2016). An implication for dominant inheritance pattern of migratory direction is that the primary mechanism maintaining a narrow migratory divide may not be the inferior intermediate route hybrid individuals take.

Figure 6. To the left a recaptured willow warbler with a removed geolocator (Photo: Harald Ris). To the right, routes of 51 willow warblers from the Swedish migratory divide color coded according to presence (red) or absence (grey) of MARB-a.

Genetics of migration timing

In paper I, we found that none of the three genetic markers associated with the timing of autumn migration. In paper V, we additionally genotyped a large number of willow warblers from across the breeding range to test whether polymorphism on the *Clock* gene will set apart the populations that all breed at different extremes of Eurasia and initiate migration at different dates but found no differences on *Clock* genotype frequencies. This is however not surprising and further solidifies the view that migration timing is an additive trait (Bossu et al., 2022).

Consequences of intermediate migratory routes (Papers II and III)

Papers II and III further analyze the geolocator data used in paper I with the expectation to identify costs for deviations from the typical eastern and western flyways.

Daytime migration

In paper II we quantified daytime migration patterns of the birds we had tracked. Willow warblers typically migrate at nights and are thought to migrate during daytime, only when encountering large ecological barriers (e.g., the Sahara Desert or Mediterranean Sea). Our expectation was that the intermediate migratory route would require the birds to migrate at daytime more often than birds that follow the typical eastern or western flyway. We instead found that it was the western flyway birds that had a higher probability to execute migratory flights in daytime, in both spring and autumn (**Figure 7**). The declining probability of daytime flights from west to east is in line with a previous result from theoretical modelling, that due to differences in wind patterns across the Sahara Desert, the western flyway is harsher and impose higher mortality on migrants (Erni et al., 2005). In addition, it has been shown that populations of the common cuckoo *Cuculus canorus* in UK that migrate more westerly are under stronger decline compared to populations that migrate more easterly (Hewson et al., 2016).
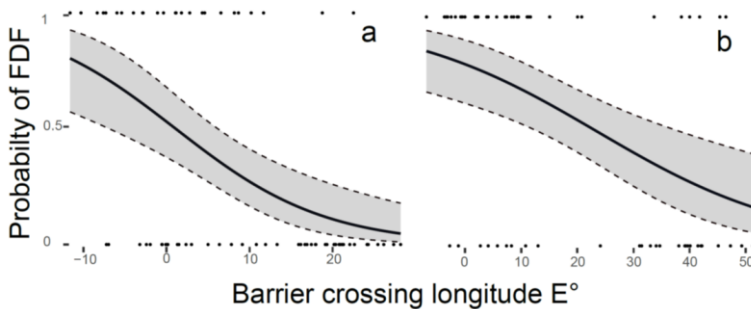
Figure 7. The probability of willow warblers doing a full day flight (FDF) in autumn (a) and spring (b) migrations relative to the barrier crossing longitudes (longitude at which birds crossed latitude 35 °N, that corresponds to Mediterranean Sea). Grey shading marks 95% confidence interval.

Loop migration patterns

In paper III we further investigated our tracking data from Sweden to study loop migration patterns in willow warblers. The term "loop migration" means that there is a substantial difference between spring and autumn routes (Newton, 2008). As expected, we found that willow warblers that follow the main flyways (western or eastern) execute counterclockwise loops. Birds that follow the western flyway use a detour following the western edge of Sahara Desert in autumn and execute a shortcut by taking a more direct route in spring. In contrast, birds that follow the eastern flyway take a direct route in autumn but carry out a substantial detour in spring flying over the Arabian Peninsula. Most of the birds that wintered at intermediate longitudes in Africa in general did not execute significant loops (**Figure 8**). A few birds that wintered at intermediate longitudes carried out clockwise loops and counterclockwise loops. We interpret this large variation in spring migration patterns as evidence that that wintering at intermediate longitudes in Africa entails less optimal options for spring migration. While birds wintering in eastern and western Africa, each have optimal routes for spring migration, wintering in central Africa impose a challenge and perhaps a survival cost. We did not find any association of the extent of the loop and any of the three genetic markers we studied.
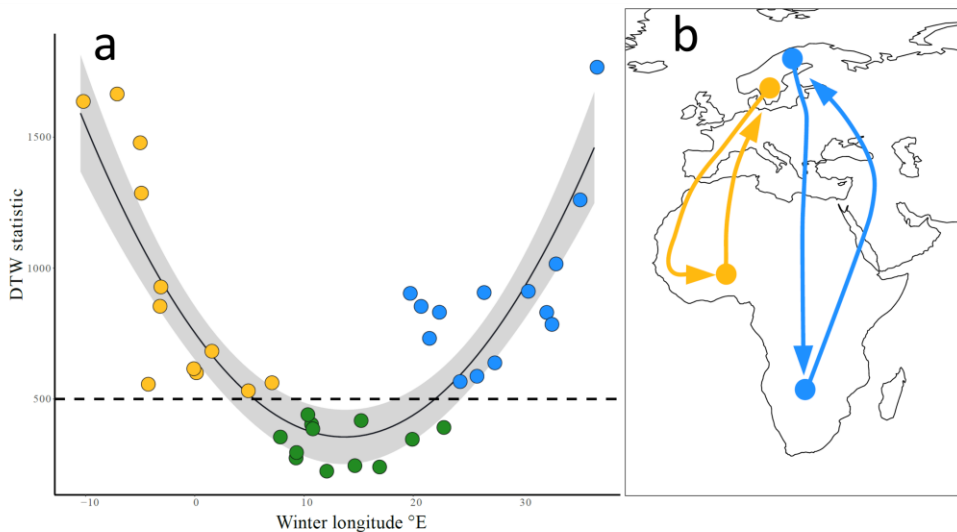

Figure 8. Dynamic Time Warping statistic quantifying differences in shape of spring and autumn routes of willow warblers relative to the wintering site longitude. The higher the DTW statistic the larger the difference between spring and autumn routes (a). Typical loop patterns of western and eastern flyway willow warblers in orange and blue correspondingly (b).

## Phenotypes and genotypes of *yakutensis* (Papers IV, V, VI)

The following three papers are dedicated to studying the far eastern Siberian subspecies *yakutensis*. In paper IV we present migratory routes of willow warblers breeding in far eastern Russia. The three birds from which we retrieved loggers begun their migration by flying NW for circa two weeks before taking a turn south to Tanzania and Mozambique (**Figure 9a**). Under a simple version of the "Clock and Compass" hypothesis, these birds should have noticeable differences in the migration direction genes compared to European *trochilus* and *acredula* that start autumn migrations with flying SW and SE, respectively. A previous study based on a 4,000 SNP array (Lundberg et al. 2017) however did not to find any genomic differences between *acredula* and *yakutensis*. In paper V we compared a larger sample of *yakutensis* with both European subspecies in terms of their genetic variation at candidate loci for migration and morphological variation. We still did not find any genetic differences between *acredula* and *yakutensis* (**Figure 9b**).
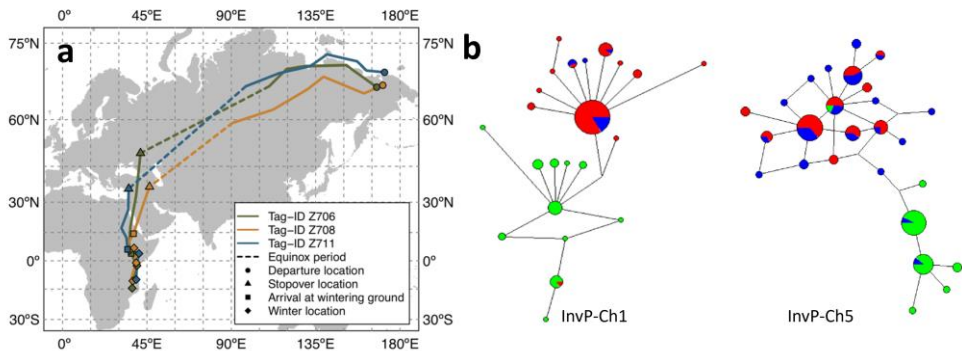
Figure 9. Migration routes of three yakutensis males tracked from eastern Siberia (a) and haplotype networks for Sanger sequenced segments of InvP-Ch1 and InvP-Ch5 (b). Each circle represents a unique haplotype. Green: *trochilus*, blue: *acredula*, red: *yakutensis*. The sizes of the circles are proportional to the frequency of the haplotype and branch lengths are proportional to divergence between haplotypes.

Our failure at first to find genetic differences between *acredula* and *yakutensis* may have been caused by ascertainment bias. Up till this point, we had focused on loci identified from comparing *acredula* and *trochilus* (Lundberg et al. 2017). During the fieldwork in eastern Siberia (Papers IV and V), we obtained additional DNA samples for genome resequencing that allowed us to compare the genomes of *yakutensis* and *acredula*. We found that the genome wide differentiation is still neglectable ($F_{st} = 0.004$). However, after investigating the most differentiated SNPs (with F_{st} above 0.7), we found that two of them were located within an exon of the *Elovl3* gene located on chromosome 6. We then took advantage of the vast collection of willow warbler skins at the Zoological Museum of Moscow state University and collected tissue samples (toe pads) of ≈ 100 willow warblers collected from breeding sites across entire Russia. We used a qPCR genotyping protocol and processed the collected material. We found that at the *Elovl3* gene, the

far east Siberian willow warblers are nearly fixed for a specific eastern allele, whereas in the western part of the range this allele occurs at a frequency of 1.5 %. Spatial genetic analyses showed that the variation of *Elovl3* changes gradually over a wide cline, with the center located around the Yenisei River (**Figure 10**). In mice, *Elovl3* is involved in fat tissue accumulation and mice with suppressed Elovl3 expression must shiver more to stay warm (Zach-Avec et al., 2010). While one needs to be cautious when extrapolating from a small rodent to a migratory bird, it is plausible that the variation on *Elovl3* in willow warblers is adaptive. Far eastern willow warblers have different migration routes, with different stopovers and breed in harsh conditions of Arctic Russia all of which possibly require adaptations different from western populations. Whether *Elovl3* is involved in migratory route choice in willow warblers will remain unclear until it is possible to track and genotype birds breeding in Yenisei region.
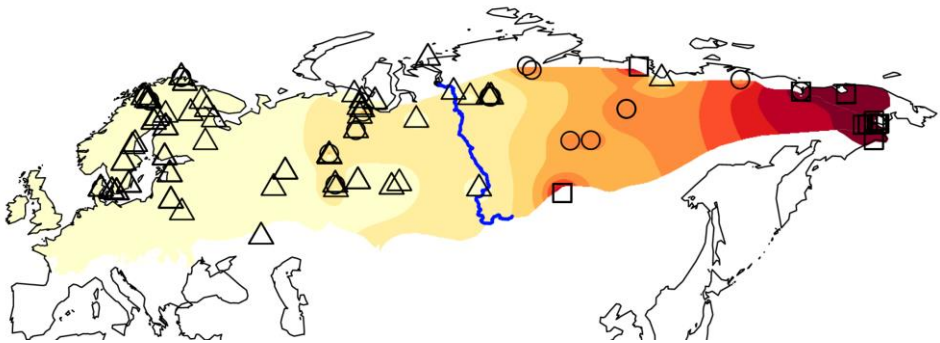
Figure 10. Extrapolation of genotype frequencies for the *Elovl3* gene within the willow warbler breeding range. The approximate breeding distribution of willow warblers used with courtesy of Birdlife International (slightly modified by KS). Squares represent eastern homozygotes, triangles western homozygotes and circles heterozygotes. The Yenisei River is drawn in blue. The locations overlap and the number of symbols therefore does not correspond to the full sample size (n=311). Color gradient from light yellow to dark red shows increasing probability of encountering *E* allele.

Closing remarks

The ultimate goal for the research field explored with my thesis is to identify how and which exact genes determine the migratory behavior of birds (**Figure 11**). So far, the results of different studies when it comes to genes associated with migration remain species specific. Lugo Ramos *et al.* (2017) attempted to separate migratory and non-migratory bird species based on 25 candidate genes with no success. This is both, frustrating and informative, however not at all surprising! Long distance migration has independently evolved numerous times across the avian tree of life. It is very likely that a single point mutation or small indel somewhere on any of the many regulatory elements involved in genetic pathways that alter the migratory behavior is enough to drastically change the entire migratory phenotype. In this scenario, mutations do not have to happen on the exact same genomic region across species, but instead must affect one or more of the relevant pathways.

It is clear that cultural inheritance and learning play a crucial role in the behaviors of long lived socially migrating species (Méndez et al., 2021; Piersma, 2011; Sutherland, 1998). The notion that songbirds such as willow warbler, truly migrate without any social influence have been challenged (Bearhop & Evans, 2019). However, since most of these migrations happen at nights and at altitudes far above of what we can observe (Sjöberg et al., 2021), we cannot directly test it yet. Nevertheless, "Emlen funnel" experiments on European blackcaps show a striking overlap with the orientation of blackcaps tracked in wild (Berthold, 2003; Delmore et al., 2020). Small songbirds typically live short lives ($\approx$1.5-2.5 years on average). I therefore think that there is very little room for natural selection to improve the capacity of learning and allowing large adjustments of migratory routes over lifespan of one individual. With annual mortality of $\approx$50%, very few individuals will have the opportunity to accumulate knowledge from multiple trips.

Ultimately, we (scientists interested in genetics of bird migration) must arrive to general conclusions. In the foreseeable future, I think it will be most fruitful to continue meticulously investigating a few already established solitarily migrating model species such as e.g., the willow warbler, the Swainson's thrush *Catharus ustulatus*, the *Vermivora* warblers and Eurasian blackcap. After we have learned more details of the inheritance patterns and shortlisted solid candidate genes in several species, we can begin to search for similarities that likely will not lay on exact orthologue genes but rather will be associated with the same pathways.

Studies that associate phenotypes with genotypes (this thesis included) do not have the power to describe proximal mechanisms of how the phenotype is determined. At the end of the day, ornithologists will have to collaborate with molecular biologists and systems biologists to carry out gene editing/knockout experiments on captive birds, preferably hatched in laboratory, free of bias from maternal effects. I think work on studying bird navigation mechanisms is crucial as well, because this

will help us to understand what are the traits of the migratory behavior that need to be measured (is it the distance, the natal inclination angle, preferred magnetic intensity gradient, or something else?). Work on the avian sensory systems will also be of utmost importance as this will allow us to better understand what information the birds are capable to perceive from all that the environment presents (this is especially important for the components of the earth's magnetic field).

Next steps for the willow warbler system

Results of my thesis show that one single locus (MARB-a) has an immense effect on migration direction. We only know that it is a large (>12 Mb) block dominated by repeated DNA elements. My fellow PhD student Violeta Caballero-Lopez currently is spearheading work on identifying the exact location of MARB-a on the willow warbler genome. After that, work can begin to investigate the genes flanking this region. Our findings after studying migratory divide in Sweden are (at least to me) stunningly clear. However, the willow warbler system presents us with two more migratory divides (one in eastern Europe and one on Åland archipelago) where *acredula* and *trochilus* meet and interbreed just as in Sweden. Tracks of genotyped birds from the two other migratory divides will show us how robust the association between MARB-a and migration direction truly is. This work has already begun by deploying geolocators on birds in Poland and Lithuania in summer of 2022 that will be harvested in spring of 2023. Work in the Åland archipelago is already planned and will start in the spring of 2023.

A limitation in studying willow warblers and all other small songbirds is that it is not yet possible to efficiently track juvenile birds on their first trip. But I do think that that the inferences we draw from studying adults will remain very relevant.

I entered the study of bird migration with a genuine sense of awe and wonder. Now that I am about to graduate with a doctoral degree, I feel an even greater sense of wonder and excitement of discoveries that lay ahead!

Figure 11. Me and Staffan somewhere in Jämtland, searching for bird migration genes. Photo: Harald Ris.

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List of Papers

I. Sokolovskis K., Lundberg M., Åkesson S., Willemoes M., Zhao T., Caballero-Lopez V., Bensch S. Migration direction in a songbird explained by two loci. (2023) Nature Communications.

II. Sokolovskis K., Caballero- Lopez V., Åkesson S., Lundberg M., Willemoes M., Zhao T., Bensch S. Day time migration patterns in willow warblers differ between the western and eastern flyway. Submitted.

III. Sokolovskis K., Caballero- Lopez V., Åkesson S., Lundberg M., Willemoes M., Zhao T., Bensch S., Loop migration patterns in Swedish Willow Warblers. Manuscript.

IV. Sokolovskis K., Lundberg M., Liedvogel M., Åkesson S., Solovyeva D., Willemoes M., Bensch S., Phenotypic and genetic characterization of the East Siberian Willow Warbler (*Phylloscopus trochilus yakutensis* Ticehurst, 1935) in relation to the European subspecies. (2019) Journal of Ornithology.

V. Sokolovskis K., Bianco G., Willemoes,M., Solovyeva D., Bensch S. & Åkesson S. Ten grams and 13 , 000 km on the wing – route choice in willow warblers *Phylloscopus trochilus yakutensis* migrating from Far East Russia to East Africa. (2018) Movement Ecology.

VI. Lundberg M., Sokolovskis K, Zhao T., Red'kin Y., Bensch S. Genomic divergence between Scandinavian and East Siberian willow warblers *Phylloscopus trochilus*. Manuscript.

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