

Charles University, Faculty of Science, Department of Ecology

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**The function and variability of song in two interacting
nightingale species**

Funkce a variabilita zpěvu dvou navzájem interagujících
druhů slavíků

Doctoral Thesis

ABEL SOURIAU

Supervisor: Tereza Petrusková

Advisor: Jiří Reif

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- AUTHOR CONTRIBUTION STATEMENT -

I declare that this thesis is my original work and has not been submitted for the purpose of obtaining the same or any other academic degree earlier or at another institution. My involvement in the research presented in this thesis is expressed through the authorship order of the included publications and manuscripts. All literature sources I used when writing this thesis have been properly cited.

My contribution to all four first-authored papers included in the doctoral thesis are as follows:

1. Contribution to the conception and design of the experiments and adaptation *in situ* to the field conditions.
2. Data collection (nightingale song recording and conducting playback experiments) in wild populations of common nightingales in Poland (Chapter 1) and thrush nightingales in Poland (Chapter 2, Chapter 4), and recording of thrush nightingales in the recent Finnish population (Chapter 3).
3. Data analysis, production of figures and interpretation.
4. Writing of the manuscript drafts, and their revision according to the input of my supervisor, co-authors, and referees (if relevant).

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Chapter 1

Souriau A, Kohoutová H, Reif J, Vokurková J, Petrusek A, Reifová R & Petrusková T (2018) Can mixed singing facilitate coexistence of closely related nightingale species? *Behavioral Ecology*, 29: 925–932.

Chapter 2

Souriau A, Reifová R, & Petrusková T (manuscript submitted) No evidence for a role of trills during territorial intrusion in a complex singer, the thrush nightingale.

Chapter 3

Souriau A, Sorjonen J & Petrusková T (manuscript draft) Song evolution after three decades in a complex songster, the thrush nightingale.

Chapter 4

Souriau A, Geberzahn N, Ivanitskii VV, Marova IM, Vokurková J, Reifová R, Reif J, Petrusková T (2019) Singing behind the stage: thrush nightingales produce more variable songs on their wintering grounds. *Behavioral Ecology and Sociobiology*, 73(11): 150.

- PREFACE -

“THE nightingale,” says Pliny, “that for fifteen days and nights, hid in the thickest shades, continues her note without intermission, deserves our attention and wonder.—How surprising that so great a voice can reside in so small a body!—Such perseverance in so minute an animal! With what a musical propriety are the sounds it produces modulated!—The note at one time drawn out into a long breath; now stealing off into a different cadence, now interrupted by a break, then changing into a new note by an unexpected transition; now seeming to renew the same strain, then deceiving expectation! She sometimes seems to murmur within herself; full deep, sharp, swift, drawling, trembling; now at the top, the middle, and the bottom of the scale! In short, in that little bill seems to reside all the melody which man has vainly endeavoured to bring from a variety of musical instruments. Some even seem to be possessed of a different song from the rest, and contend with each other with great ardor. The bird overcome is then seen only to discontinue its song with its life.”

from *Natural History of the Nightingale* by John Legg (1779), citing Pliny the Elder, *Historia Naturalis* lib. X chap 29 (77 AD).

My own encounter with the nightingale (or rather the two “sisters” Procne and Philomela), along the riverbanks of Poland was so special in many ways. But better than trying to put this in words I can only exhort the reader to go and spend a vibrant spring night listening to the grand voices of those little birds.

There is there all the beauty the soul needs, and surely more than enough biological mysteries to feed a scientist curiosity for years.

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Thanks and best of luck to the dear colleagues from all over the world I met during all those intense and fascinating conferences, I am sure that many of you will shape a bright future for bioacoustic research. Thanks Alexandra of the crow, for giving back the keys to those who actually sustain and use scientific knowledge.

Though they deserve far more, I'll address a big děkuji mockrát to all my friends and colleagues that made Viničná a place of life for the last 6 years. During those long years many of you ended up being a source of inspiration one day, an unexpected helping hand the other, or the little hint of good spirit that everyone needs to keep up. So cheers to the Thai food crew, the tea-time companions, and all the joyful Ryba's comrades!

Merci aussi à mes parents, qui ont eu la patience de voir leur plus-si-jeune fils mener une vie de bohème à travers l'Europe et de s'émerveiller encore de mes histoires d'oiseaux.

Congrats to my brother that ended up being a doctor before me, in topics of sustainability that could only make an ecologist proud.

My last thanks go to the birds, as I witness their arrival from exhausting migration in the freezing morning, their struggle to find love and the best home for it, all for the privilege to pass on their genes to the next generation of feathery music-boxes marvels.

Their bravery shall be remembered.

“Je sers la science et c'est ma joie” – *Basilus simplex* (Turk & DeGroot 1977)

- ABSTRACT -

The melodious and complex song of nightingales is a well-established model in studies focussing on the development and function of birdsong. Moreover, two sister species: the thrush nightingale (*Luscinia luscinia*) and the common nightingale (*L. megarhynchos*), meet in a recent secondary contact zone in Central Europe, in which their close ecological preference result in competitive interactions and interspecific hybridization. In sympatry, thrush nightingale males often replace part or all of their song repertoires with the songs of the common nightingale, a phenomenon called “mixed singing”, while the opposite tendency has not been observed. Understanding the reasons behind the occurrence of thrush nightingale mixed singing, as well as exploring the similarities and difference in song structure between the two species, were the main aims of this thesis.

In the first chapter, we tested the role of mixed singing on breeding common nightingale males and revealed its possible function in mediating territorial conflicts. Such advantage might be adaptive as it could help balance the potential costs of heterospecific song copying, and therefore help maintain the asymmetric song convergence between those species. The second chapter aims to explore further the function of thrush nightingale song structures. Since broadband trills in common nightingale songs are candidate structures for aggressive motivation signalling, we tested the role of the similar broadband “rattling” phrases in thrush nightingales, by comparing the song of breeding males before and after a simulated territorial intrusion. As no significant difference was found, we propose that similar structures might have different roles between the two study species.

The second part of this thesis arises from the several international collaborations developed during my PhD study. The third chapter takes advantage of a sample of historical recordings to explore the change in thrush nightingale song repertoire over time, by comparing them with recent recordings from the same location in Eastern Finland, three decades later. Our results showed a complete turnover of song types, while 40% of old syllable types were still present in recent repertoires. This first insight on long term change in species with a complex song suggests that only part of the song repertoire is maintained while other levels of song organisation are eventually modified. Focusing on the variation of song between seasons, the last chapter explores for the first time the songs of thrush nightingales at their African wintering ground. This study confirmed a substantially higher song plasticity in the wintering population compared to the European breeding ones, in both syllable consistency and element versatility. These results support the hypothesis that winter singing plays a role in song improvement, paying off during the next breeding season. The work of this thesis highlights the importance of combining different approaches to investigate such complex traits as birdsong, in exploring the many levels of song organisation as well as the parts of life cycle overlooked in previous research.

- ABSTRAKT (in Czech) -

Melodický a složitý zpěv slavíků je dobře zavedeným modelem pro studium vývoje a funkcí ptačího zpěvu. U dvou sesterských druhů tohoto rodu, slavíka tmavého (*Luscinia luscinia*) a obecného (*L. megarhynchos*) dochází v recentní zóně sekundárního kontaktu ve střední Evropě k jevu nazývanému „smíšený zpěv“, kdy slavíci tmaví vkládají do svého repertoáru části nebo i celé zpěvy slavíka obecného. Opačným směrem k přebírání zpěvů nedochází. Oba druhy slavíků mají podobné ekologické nároky a v oblasti jejich kontaktu lze pozorovat jak mezidruhové střety, tak případy mezidruhového křížení. Hlavním cílem této disertační práce bylo přispět k objasnění důvodů, které vedou k existenci a přetrvávání smíšeného zpěvu u slavíků tmavých a zároveň prozkoumat i další aspekty zpěvu tohoto druhu, který je oproti zpěvu slavíka obecného probádán mnohem méně.

V první kapitole jsme testovali roli smíšeného zpěvu za pomoci playbackových pokusů na samcích slavíka obecného a odhalili jeho možnou funkci při mezidruhových teritoriálních střetech. Zdá se, že smíšený zpěv by mohl zmírňovat přímé agresivní konflikty, což by mohlo být natolik výhodné, že to vyváží i potenciální negativní dopady kopírování heterospecifických zpěvů. Takovýto proces by mohl umožňovat zachování asymetrické konvergence zpěvů mezi oběma druhy. Druhá kapitola disertační práce si klade za cíl prozkoumat další funkce zpěvu slavíka tmavého. Protože trylky s velkým frekvenčním rozsahem signalizují ve zpěvech slavíků obecných agresivní motivaci, zaměřili jsme se na podobné trylky v „kastanětových“ frázích zpěvu slavíka tmavého. Porovnávali jsme spontánní zpěvy samců nahrané před simulovaným vniknutím cizího zpívajícího samce do jejich teritoria se zpěvy z reakce na playbackovou nahrávku. Žádné významné rozdíly jsme však nenalezli, předpokládáme tedy, že podobné struktury ve zpěvu mohou mít i u takto blízké příbuzných druhů odlišné role.

Druhá část této disertační práce vychází z několika mezinárodních spoluprací, které vznikly během mého doktorského studia. Třetí kapitola využívá historické nahrávky k prozkoumání toho, jak se změnily zpěvy slavíka tmavého na téže finské lokalitě s odstupem více jak třiceti let. Výsledky ukázaly, že zatímco u typů zpěvů došlo k úplné proměně, 40 % typů slabik zaznamenaných před třemi dekádami bylo stále přítomno v současných repertoárech místních slavíků. Obdobných studií studujících dlouhodobé změny ptačího repertoáru není mnoho a většina se zaměřila na druhy s jednoduchým zpěvem. Naše výsledky ukazují, že druhů se složitým zpěvem je třeba brát v potaz různé úrovně, na kterých se zpěv vyhodnocuje: zatímco komplexnější struktury mohou úplně vymizet, základní jednotky, ze kterých je komplexní zpěv tvořen, mohou přetrvávat i velmi dlouho. Poslední kapitola se zaměřuje na rozdíly ve zpěvech slavíků tmavých na hnízdišti a zimovišti. Řadí se tak k několika málo studiím, které se zabývají zpěvem migrujících druhů mimo hnízdní období. Tato studie potvrdila podstatně vyšší plasticitu zpěvu v africké zimující populaci ve srovnání s hnízdními populacemi v Evropě, a to jak na úrovni konsistence slabik, tak i na variabilitě elementů. Výsledky podporují hypotézu, že zimní zpěv hraje roli při zlepšování zpěvu a tento „zimní trénink“ se může vyplácet během příštího období rozmnožování. Studie zahrnuté v této práci, kombinující různé metodologické přístupy, přinášejí nové vhledy do studia komplexního ptačího zpěvu mimo jiné díky zaměření na úroveň organizace a části životního cyklu v předchozím výzkumu opomíjené.

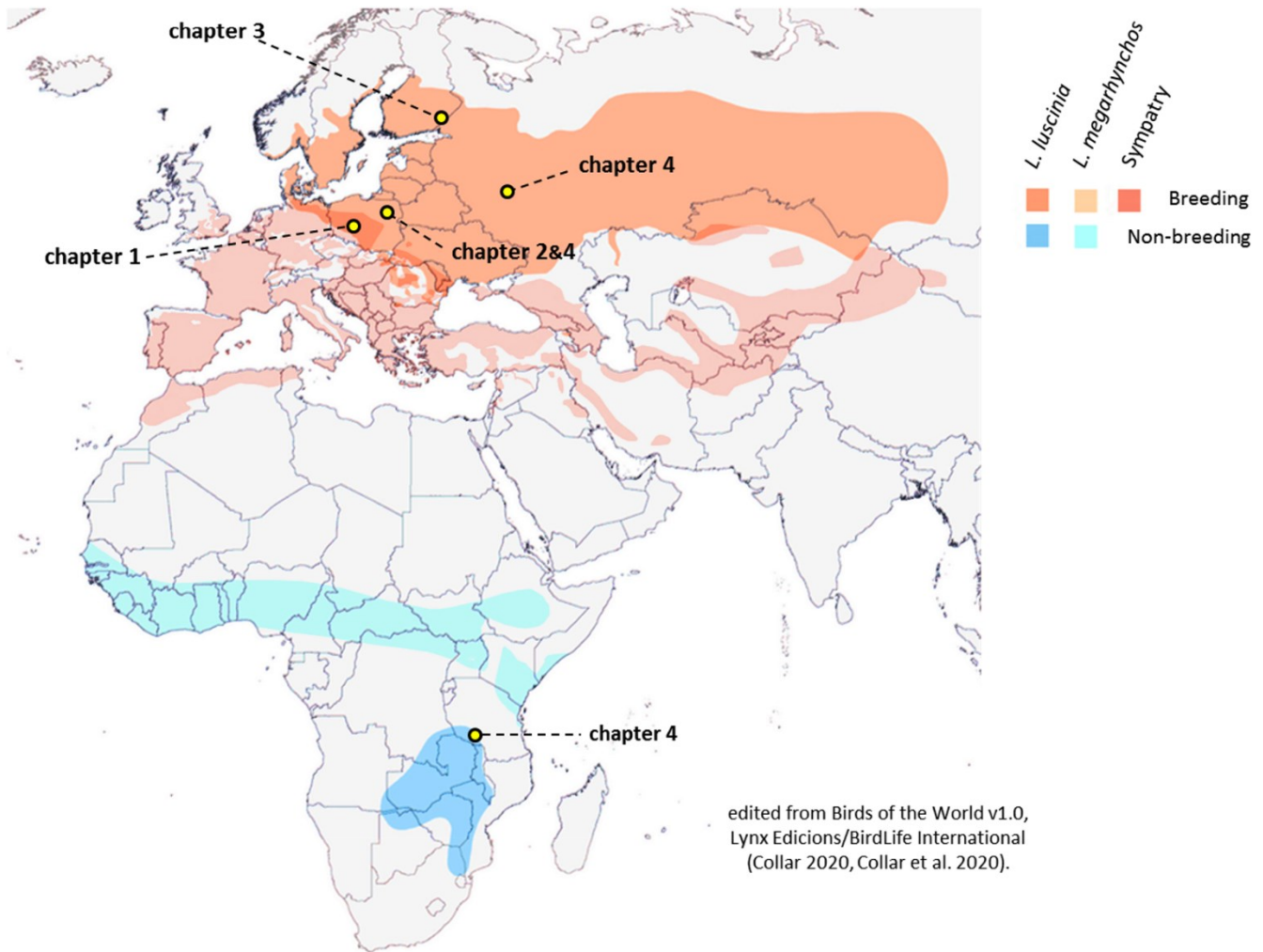


Figure 1 – Distribution area of the two studied species of nightingales at both their breeding and wintering grounds (see legend). The thrush nightingale (*L. luscinia*), in bold colours and the common nightingale (*L. megarhynchos*), in light colours, meet over sympatric zone (darker red) as the one crossing central Europe. The location of the different populations studied in this thesis are displayed along with the reference to their related chapters.

- GENERAL INTRODUCTION -

The virtuosity of birdsong has caught human imagination and curiosity for ages. From the early naturalist work of Pliny the Elder in his *Naturalis Historia* during the 1st century to classical poets like William Shakespeare, the song of nightingales, among others, always inspired. Centuries later, in *The descent of man and selection in relation to sex*, Darwin (1871) gives to birdsong a prominent place, as an example of secondary sexual trait in the elaboration of his sexual selection theory. In the light of this evolutionary perspective, scientists have been relentless in their effort to understand the mechanisms and functions explaining the existence of such an elaborate trait.

The story of bird song starts with a special sound-producing organ, the syrinx, found for the first time in an ancient bird around 68 Mya (Clarke et al. 2016). The syrinx is a bipartite structure located at the tracheobronchial junction and composed of a pair of labia on each side, which vibration allows either lateral or bilateral phonation (Suthers & Zollinger 2004). One group in particular, the songbirds (oscine), has been shown to evolve unprecedented morphological features providing them with outstanding song abilities (Gaunt & Nowicki 1998, Riede & Goller 2014). Accordingly, the songbirds' syrinx is also composed of five pairs of syringeal muscles, each playing an important role in controlling different parameters of the song, as for example the timing or the frequency of sound produced (Larsen & Goller 2002). Such a precise coordination of respiratory and vocal organs also requires a complex neural system to allow the high degree of precision required to produce species-specific songs (Suther et al. 1999). Additionally, another important feature of the neurological evolution in oscine birds is the ability to learn and refine songs, only made possible by the development of specialized forebrain areas, in which motor and auditory centres interact closely (Doupe & Kuhl 1999). As a consequence, the transmission of song by imitation implies that songs are transferred across generations as a cultural trait (Slater 1986). Cultural selection can thus be considered as an evolutionary force on its own, while still interacting with the genetic bases determining the filters for conspecific song recognition and learning (Lachlan & Slater 1999).

In some cases, however, birds are also found to imitate sounds out of their conspecific models. Vocal mimicry, for example, refers to the capacity of some birds to copy vocalisations from other species, often targeting simpler “calls” and generally used out of any appropriate context (Hindmarsh 1986, Garamszegi et al. 2007). Despite exploring several promising hypotheses such as predation avoidance, social parasitism or intraspecific sexual selection, Kelley et al. (2008) concluded that no evidence was strong enough to generalize a functional explanation for the occurrence of mimicry, and rather considered it as a

learning mistake. Nonetheless, several examples of functional mimicry were also documented in particular species, such as the ones found in drongos: Greater racket-tailed drongos (*Dicrurus parasideus*) were shown to use mimicry to attract other species into mixed species flocks, improving their own foraging benefits (Goodale & Kotagama 2006). Another spectacular example was shown in the kleptoparasitic fork-tailed drongo (*Dicrurus adsimilis*): birds were able to perfectly mimic the alarm calls of target species, from other birds, but also meerkats (*Suricata suricatta*) and used them as a deception strategy to help steal the copied species' food (Flower 2010).

More rarely, some species are even found to thoroughly imitate the songs of another target species and fully integrate them in their repertoire in a process called “mixed singing”, likely doing so through their early song learning process (European species reviewed in Helb et al. 1985). Unlike simple mimicry, mixed singing only focuses on closely related species. It is usually asymmetrical, and the proportion of copied songs may vary a lot between individuals of the same population (Qvarnström et al. 2006, Vokurková et al. 2013). While also primarily thought to result from misdirected learning mistakes (Helb et al. 1985) and to be maladaptive by favouring hybridization (Haavie et al. 2004, Qvarnström et al. 2006), some studies suggest a functional role of mixed singing in improving the communication with interspecific competitors (Gorissen et al. 2006, Reif et al. 2015). In **Chapter 1**, we investigated such a role in two species of European nightingales: the thrush nightingale (*Luscinia luscinia*), often performing mixed singing when co-occurring with its closest relative, the common nightingale (*L. megarhynchos*) (Sorjonen 1986, Vokurková et al. 2013). From an evolutionary perspective, we assume that, despite its cultural aspects, mixed songs may constitute an actual example of convergent agonistic character displacement. Among other criteria, it does not simply result from hybridization (Vokurková et al. 2013) and as a territorial signal could affect the intensity of interference competition between the two species. A first study on heterospecific territoriality between the two species showed that in both species the males were actively reacting to the presence of the other (Sorjonen 1986). However, as the playback stimuli were always presented to the tested males with the same order and between short intervals of time, we cannot exclude a potential bias on the individual response. Another study from Reif et al. (2015) showed that common nightingale, the copied species, reacts significantly more to conspecific playbacks compared to heterospecific ones, while thrush nightingale reacted to both equally. In order to get insights on the potential territorial role of mixed singing, we thus aimed to test the territorial reaction of common nightingales to simulated intruders singing the songs of either species as well as mixed ones (methodological details in **Chapter 1**). Interestingly, mixed singing stimulations provoked an intermediate

composite response: first of all, as expected, the common nightingale reacted more to conspecific songs, but also showed an intermediate level of vocal response to the mixed stimulus. Mixed songs thus involved a medium counter-singing response that might reflect the partial presence of its own species songs in the playbacks. Nevertheless, instead of being simply misled in species recognition, our results suggest that the tested males were able to discriminate the singer's species, as their physical response to mixed singing (including the closest approaches and risky aggressive displays) did not differ from the response to pure thrush nightingale songs. Common nightingale thus seems to recognize and react to a competitor while still recognizing the singer's species and avoid a costly confrontation with its bigger cousin. Mixed singing, while likely originating from misdirected song learning, could nonetheless constitute a functional signal toward heterospecific competitors with adaptive value in regulating competition by establishing clear territorial boundaries, as already suggested in other species (e.g. Gil 1997). From an evolutionary point of view, and despite being only indirectly linked to genetic bases as it is transferred culturally, mixed singing may thus be considered as a case of convergent agonistic character displacement as predicted between two species involved in interference competition.

Nevertheless, to fully understand the role of birdsong in signalling, we need to consider the diversity and variation of its components, as well as the syntactical rules that shape it. Bioacousticians generally define the smallest detectable song components as elements, often combined in repeatable syllable units, themselves constituting the phrases of a song. The existence of variations in the various song components and their sequencing suggests that birds possess functional mechanisms to identify, categorize and combine sounds (reviewed in Ten Cate 2014). For example, common starlings (*Sturnus vulgaris*), have shown the ability to recognize recursive acoustic patterns out of strict syntactical context, in a similar manner to humans (Gentner et al. 2006). As a consequence, birds may use different song structures as a variety of distinct song signals.

In our temperate latitude, birdsong generally refers to the conspicuous vocalisations of males during the breeding season and is mainly used for territorial maintenance and mate attraction (Catchpole & Slater 2008). Competition between males eventually leads to the signalling of aggressive motivation, predicting an escalation in conflict (Searcy & Beecher 2009). Aggressive motivation signalling has thus often been associated with an increase in the rate of songs (Baker et al. 2012, Petrusková et al. 2014), syllables (Linhart et al. 2013, Funghi et al. 2014) or elements (Podos 1997, Vehrencamp et al. 2012). In common nightingales, in particular, aggressivity has been linked to the use of fast-element structures such as broadband trills (Kunc et al. 2006, Schmidt et al. 2008, Sprau et al. 2010). Since in thrush nightingale the

trill structures are a most common and diversified feature, characteristic of the song ending (Sorjonen 1983), it may also play a role in similar contexts. In **Chapter 2**, we investigated the potential role of trill for territorial signalling in male thrush nightingales, by comparing the trill use and their temporal parameters between spontaneous song and response of a tested male to a playback simulating a conspecific intrusion. Contrary to our expectations, while all the birds reacted to the playback, by counter-singing and displaying various degrees of investigation, no significant change in the type of used trills or the modification of trill length or rate was detected. Moreover, those parameters did not differ between the boldest birds, reacting fast and taking the most risk to approach the intruder, and the ones carefully staying inside the vegetation cover and reacting late. Altogether, those results did not support a role of trills in territorial interactions in thrush nightingale, contrasting with the use of similar structures in its cousin species, the common nightingale.

Another approach to address the matter of song variability would be to explore the maintenance of bird song components and syntax over long periods of time. Due to the logistic constraints and technological limits in the past, long-term study are usually limited in time and still remain scarce (e.g. Ince et al. 1980, Derryberry 2009, Ju et al. 2019). Interestingly, changes in vocal repertoires over a long period of time seem to vary a lot depending on the study, with examples showing surprisingly stable song types over decades in some species (Goodale & Podos 2010) whereas others illustrate a complete song repertoire turn over (Holland 1996). Altogether, these results suggest that long-term studies may inform on the potential differences in cultural transmission and song maintenance mechanisms existing between species. Supporting this idea, a study of Harbison et al. (1999) on the song repertoire in white-crowned sparrows (*Zonotrichia leucophrys oriantha*), suggest that the contrast in song evolution rate over 26 years between two populations was directly linked to the populations size and habitat discontinuity. The study presented in **Chapter 3** took advantage of the archive of historical thrush nightingale song recordings deposited in the Natural history museum of Helsinki for more than 30 years. In this chapter, we explored the long-term change in the repertoire at both the song type and syllable type levels, and their sharing among individuals at the same locality in Finland between 1986 and 2019. Our analysis of song repertoires firstly revealed that no song types were shared between the old and the recent population, suggesting that the maintenance of strict syntactic rules defining a song by its syllable associations could be difficult. Moreover, the conservation of a proportion as high as 40% of the syllable type repertoires from 1986 after more than three decades proves that simpler song structures can be maintained for a long period of time, even in complex singers such as our model species.

Nonetheless, some other aspects of song variability might also be addressed on a shorter time scale, taking advantage of the different contexts in which the birds sing. Following this idea, the last chapter of this thesis aims to explore yet another rather overlooked side of the nightingale singing behaviour. From its early development historically based on northern temperate zones, the studies of birdsong have largely focused on the conspicuous song of males and its functions during the most active breeding period (e.g. Catchpole 1987, Kroodsmas & Byers 1991). While the recent years showed a renewed interest for female song (see Odom et al. 2014, Riebel et al. 2019), our knowledge on the occurrence of song in long-distance migrants out of their breeding ground still remains very scarce. Following a pioneering study on European bird wintering in Africa from Kelsey (1989), that favoured a territorial function to secure food resources, a study of Sorensen et al. (2016) hypothesised a potential role of winter singing in the song development process. Subsequent work from Kipper et al. (2016) on the common nightingale, while not showing strong differences in songs from breeding season compared to the wintering ones, also found a lack of song repetition that could support a role in song rehearsal. To complement these first studies, our study presented in **Chapter 4** focused on the difference of song in the thrush nightingale between a wintering population from Tanzania and two breeding populations from Europe. Based on the important plasticity revealed by a preliminary exploration of the wintering songs, we decided to base our analysis at two fine structural levels of the song - syllables and elements. By the use of semi-automated cross-correlation, we showed an important lack of consistency in syllable repetitions over different songs of the same type. The irregularity of song was also confirmed by the estimation of element versatility by several observers, showing an exaggerated number of different notes compared to the more stable element sequencing in breeding songs. These results highlight some important similarities between our observation from the field and the phase of song plasticity as observed for example in common nightingales (Hultsch & Todt 2004). This phase of song development plays an important role in song rehearsing, before crystallizing a more stable song repertoire that will determinate the male's song performance during the next breeding season. It might even, to some extent, provide insight on the processes of song imitation and refinements responsible for establishment of male repertoire and thus mixed singing in the relevant populations.

- NIGHTINGALES AS A MODEL STUDY -

The thrush nightingale *Luscinia luscinia* (Linnaeus 1758) and the common nightingale *Luscinia megarhynchos* (hereafter referring to the nominal subspecies *L. m. megarhynchos*; Brehm 1931) are two small migratory songbirds of the Muscicapidae family breeding across the northern part of the Palearctic region. The two species show no sexual dimorphism and look rather similar, with thrush nightingales being sensibly bigger in size and common nightingale having a brighter plumage along with a reddish rump and tail (Cramp & Perrins 1994, Collar 2020, Collar & Christie 2020). While inconspicuous coloured, both nightingale species are characterized by the active male singing during the first stages of the breeding period, performing by night and day (Sorjonen 1977, Cramp & Perrins 1994).

The two species are closely related and diverged about 1.8 Mya during the Pleistocene (Storchová et al. 2010) before recolonising different parts of the western Palearctic. They currently meet over several secondary contact zones, among which is a well-documented area across central Europe (Sorjonen 1986, Kováts et al. 2013, Vokurková et al. 2013, **Chapter 1, Figure 1**). The two species occasionally hybridize (Becker 2007, Kverek et al. 2008, Reifová et al. 2011, Kováts et al. 2013), but only a low proportion of hybrids are found in those populations, suggesting the existence of incomplete reproductive barriers (Reifová et al. 2011, Albrecht et al. 2019). Both species show very similar ecological preferences (Cramp & Perrins 1994), beside an inclination for drier habitats in common nightingales or wetter ones in thrush nightingales, likely responsible for a partial habitat and potential diet segregation in sympatry (Sottas et al. 2018, 2020). Competition over territory in their secondary contact zone thus leads to aggressive territorial interactions between them, more especially in the form of singing response (Sorjonen 1986, Reif et al. 2015, **Chapter 1**). Moreover, the co-occurrence of the two species often results in the inclusion of common nightingale songs in thrush nightingale repertoires, providing a good model species to study mixed singing (Sorjonen 1986, Vokurková et al. 2013, **Chapter 1**). Although the song of the two species retains similarities compared to other related species, they arguably differ in structure and repertoire: common nightingales are characterized by shorter song and faster song rate, an individual repertoire usually containing several hundred of song types (from around 150 to 280) (Hultsch & Todt 1981, Kipper et al. 2004), with no strong variation across its distribution area. As a contrast, thrush nightingale songs are usually of longer duration, separated by longer pauses and individual repertoires are way smaller, varying from around 10 to 40 song types depending on the study (Sorjonen 1987, Griessman & Naguib 2002, Marova et al. 2015). Thrush nightingale songs are also known to vary geographically (Sorjonen

1987, Marova et al. 2015, **Chapter 4, Figure 2**). Mixed singing between these species thus results in thrush nightingale males either integrating a variable proportion of clearly identifiable heterospecific songs (from a few songs up to the totality of a bird repertoire, at least on the short term, Vokurková et al. 2013) or possibly the inclusion of common nightingale song components into the classical species syntax (**Chapter 1**). Additionally, the two species also differ in migrating habits (Cramp & Perrins 1994): if the thrush nightingale meet with the nominal subspecies of *L. megarhynchos* at their breeding ground, their migrating path overlaps during a significant period of time with the one of the two other subspecies *L. megarhynchos africana* and *L. megarhynchos golzii* in eastern and southern Africa (Pearson 1984).

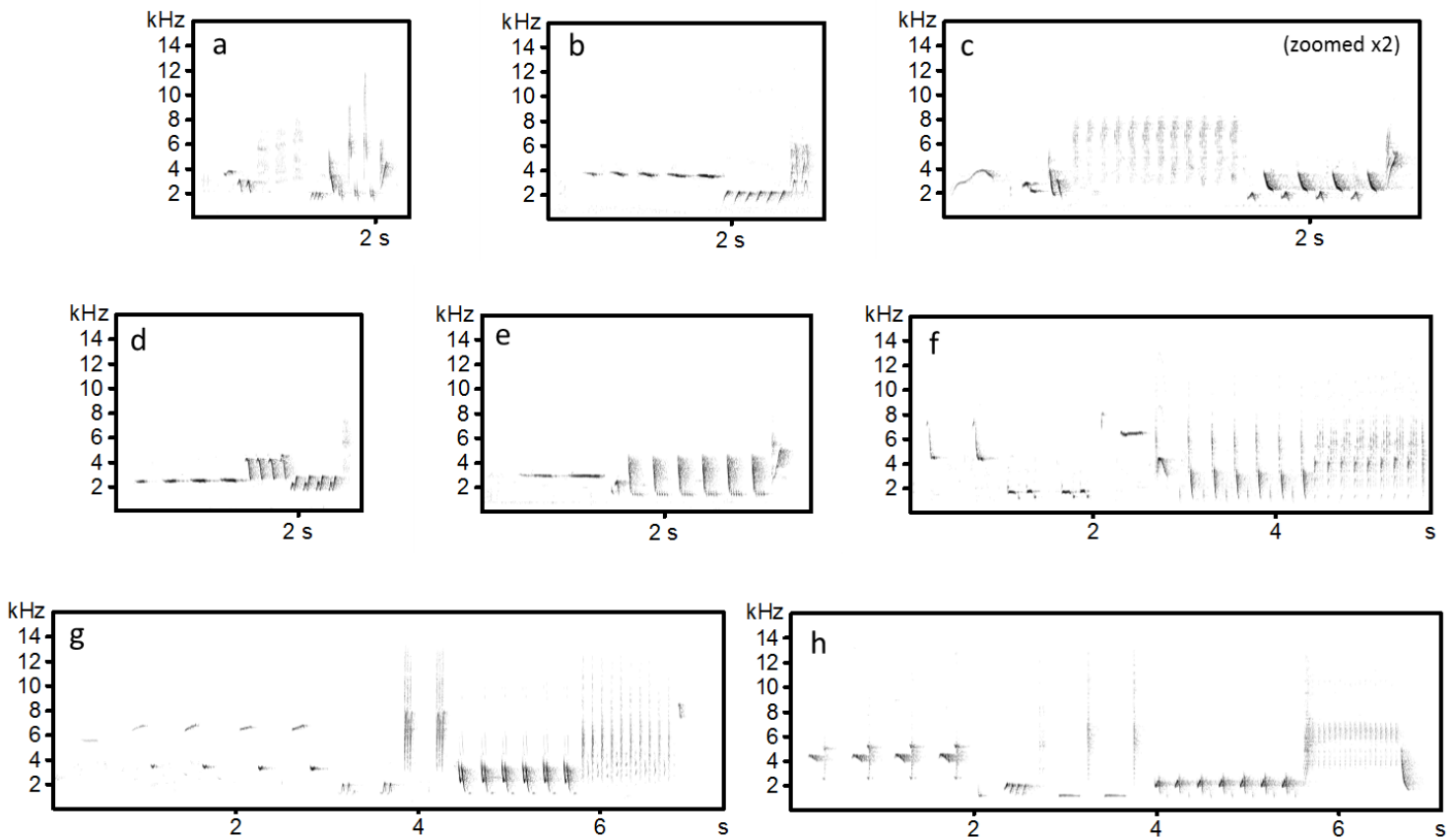


Figure 2 - Song examples I recorded from various studied populations included in this thesis. The first row (a,b,c) shows songs recorded from common nightingale in southern Poland, 2016. The second row displays mixed songs (d,e) or conspecific songs (f) recorded from thrush nightingales at the same location during the same year. The last line shows songs from allopatric populations in northern Poland, 2016 (g) and Finland, 2019 (h). All the spectrograms display the frequency (kHz) on time (s).

- CONCLUSION & FUTURE PROSPECTS -

Our studies improved the understanding of several mechanisms related to the occurrence of the still controversial adaptivity of mixed singing, while also contributed to the knowledge on the function and variability of song structures in thrush nightingales. However, many aspects of these topics still need to be addressed. This thesis might therefore also provide insights for potential future studies.

Although mixed singing is not a result of hybridization (Vokurková et al. 2013), thrush nightingales may not consider that the songs of common nightingale are ‘alien’ to their species. Both the undifferentiated territorial response to hetero- and conspecific song playbacks and the lack of learning filters suggests so. Mixed singing might thus be a signal directed to the copied species, which discriminates between the two species songs, as now finally confirmed by the territorial responses of common nightingales to mixed singing revealed in **Chapter 1**. Whether mixed singing is adaptive for the males should depend on the social context. In southern Poland, I personally observed the two species interacting a lot, resulting in frequent counter-singing and chasing. In such a population, mixed singing could be adaptive as it may reduce the costs of competition and confer an advantage to the males. Nevertheless, in several cases male thrush nightingales were found to sing heterospecific songs to an audience of conspecific neighbours only, and vice-versa. This suggests that on the one hand, mixed singers cannot flexibly switch between conspecific and heterospecific songs, which makes sense as thrush nightingale does not seem to discriminate between species-specific songs. On the other hand, this highlights that the mechanisms determining that a bird repertoire most likely depend on its past experience, and that studies focusing on song learning and long-term monitoring of individual repertoire change are crucial for this matter.

To answer the question of the maladaptive or adaptive value of mixed singing, we would need to primarily investigate its costs. Given the importance of a song-specific signal, and even though it may vary a lot over time as suggested in **Chapter 3**, replacing part or all of an individual song repertoire by heterospecific songs should not be without consequences. The results of our study on trills in **Chapter 2** suggest that similar song structures in either species might not be used for the same functions by the other, supporting the hypothesis of a cost of mixed singing by a loss of signal in conspecific interactions. Nevertheless, considering that a particular song structure would only play one unique role may underestimate the subtlety of complex song signalling. While the final trills in **Chapter 2** study did not reveal any significant link to stimulation or strategy of response, it was noted that the longest and fastest trill types were all sung by bold males. While this supports a correlation between potentially costly trills and male quality, it also suggests that we might overlook fine structure signalling when only considering a full trill category, or the relationship with only one parameter of it. For example, a growing number of studies based their approach on the vocal deviation (Podos 2001), a composite measure of the temporal and frequency structure of a song. Nonetheless, this approach being highly sensitive to the bandwidth detection, we preferred to use alternative parameters to study our wild populations.

Aside from male-male interactions, another important aspect of song convergence with a co-occurring and closely related species is also the risk of hybridization. Indeed, birdsong often serves as an important breeding barrier between related species. Focusing on the sexual selection, female preference for pure or

mixed songs might thus play a particularly important role in the evolution and maintenance of this phenomenon. In nightingales, the low occurrence of hybrids already suggests the existence of strong pre and/or post zygotic barriers. Following Haldane's rule predicting a higher genetical burden for the heterogametic sex, the sterility of hybrid female offspring constitutes a strong cost for a mixed singing male fitness (Reifová et al. 2011). Experiments on female preference for songs in controlled environments have already been successfully achieved in common nightingales (e.g. Bartsch et al. 2016). Nonetheless, in preliminary experiments using a similar approach in our wild population of thrush nightingales, we did not manage to control for the female stress and unfamiliarity with the experimental settings. While maintaining a captive population of thrush nightingale might prove difficult, we advise future studies on mixed singing adaptiveness not to overlook the importance of female preferences and mate choice.

Another promising approach to understand how mixed singing originally appeared would be to address thoroughly the process of song learning that leads to the formation of an individual repertoire. The degree of precision with which thrush nightingale imitate common nightingale songs suggest that songs were copied directly from their sister species, and not from several generations of other mixed singers. In contrast, the occurrence of a lower number of songs combining syllables of the two species into "hybrid" song types (**Chapter 1**) might originate from two processes: the improvisation of a new recombined song type by mixed-singing males, or the possible integration of mixed song elements through an imperfect transmission across a greater number of generations. To understand the factors favouring or constraining the copy of heterospecific songs, one may thus try to connect the acoustic environment of a young bird with its vocal performance during the next years. Such an approach, aside from using captive populations, may also be realized in the field by ringing nestling at one breeding location and see how the birds perform in the next breeding season, given that male in this species are highly philopatric. However, the access to nest and nestling is particularly difficult due to the cryptic habit of those species. Unsurprisingly none of the birds recorded during wintering in **Chapter 4** showed mixed song elements, as most thrush nightingale populations are allopatric to the common nightingale. Nonetheless, the miniaturisation of GPS and geolocator technologies may allow the tracking of same individuals over long distance, even in small passerine birds such as thrush nightingale (see e.g. Stach et al. 2012). It should be soon possible, with a large sampling effort, to collect information on the soundscapes of one particular individual from its breeding ground to its wintering ones. A comparison of the surrounding songs heard during the potential practicing after migration with the next-season performance would greatly improve our understanding on how those complex songs are filtered, refined and then used by thrush nightingales.

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- OUTLINE OF PUBLICATIONS & MANUSCRIPTS -

This thesis consists in four chapters, focusing on different aspects of the singing behaviour in two species of nightingales. It is subsequently divided in two complementary parts, as follows: the first part investigates the function of song in territorial conflicts, using a combined acoustic and behavioural approach. In the second part, I explored the song variability in the thrush nightingale, at different levels of song syntax. All the chapters included in this thesis were first-authored (among which one first authorship is shared). Two are published in international peer-reviewed journals, one is under review and a last one will be submitted soon after the defence.

Chapter 1 reflects the early focus of this project, with an aim to explore the phenomenon of mixed singing, and its potential role in interspecies communication. Taking advantage of an already studied population of thrush and common nightingale living in sympatry, we tested the territorial reaction of male common nightingales to simulated intrusions of another male singing conspecific songs, heterospecific songs or mixed songs (i.e. containing both heterospecific and conspecific songs copied by a thrush nightingale). The results of this study revealed that the tested males showed a similarly low level of physical response between the two heterospecific intrusion treatments, suggesting no misleading effect of mixed singing in interspecies recognition. As a contrast, we observed an intermediate level of counter-singing response to mixed songs, in between the lower heterospecific and higher conspecific degree of song response. This suggests that the maintenance of mixed singing may be linked to a role in improving the communication in territorial conflicts between two closely related species, mediating the cost of their interference competition.

Chapter 2 comes from the realization that to understand deeper the potentially functional signalling between those species, we first have to know more about their function within their own species. While common nightingale was a prolific model species in studies on song development in captivity and those discovering the role of its song structures and strategies in the wild, its cousin, the thrush nightingale, received less attention. A good candidate structure to base our study on would be trills, as it is already known to play a role in territorial responses in its sister species. The study presented in this chapter thus focused on the potential contrast in trill used in songs of tested males before and after being provoked by the simulation of another male's intrusion through a playback experiment. While all birds reacted to our stimulation, no difference was reflected in neither the frequency of trill use nor a change in the trill rate. These results suggest an alternative role for trills structure and parameters that we tested, such as signalling

a male quality over its immediate aggressive motivation. Additionally, it also highlights the complexity of considering song communication between two species with potentially different signalling for the same type of song structure.

Chapter 3 was the result of an international collaboration with the Natural museum of Helsinki, that allowed us to address the matter of the maintenance of song diversity and structure over a long period of time. We compare old historical recordings of thrush nightingales with new ones from my last field season, recorded 33 years later and the same locality in Eastern Finland. Due to the expected difference in complexity and learning constraints between various syntactical levels, we estimated each bird's repertoire at both the song type and syllable type levels. The results confirmed our predictions for an important change of repertoire over time, but also revealed a complete turn-over of song type repertoire over three decades, despite the tolerance of our approach for some degrees of dissimilarities. Nevertheless, around 40% of the syllable types from the old population was still used by the males recorded in 2019 showing that, to some extent, song structures can be selected to last over many generations. The question if those changes are shaped by female preferences, the acoustic constraint of the habitat or are a more indirect consequence of population dynamics will need further exploration.

Last but not least, **Chapter 4** investigates the rather overlooked phenomenon of migratory birds singing out of the breeding seasons. Taking advantage of multiple international collaborations, the study presented in this chapter compared the song of thrush nightingales from a wintering location in Tanzania with two unrelated breeding populations, from Poland and Russia. Following the first observations from the field and enlightened by several insights from song development studies in captive populations, we predicted a stronger song plasticity in the wintering population compared to the breeding ones, in both consistency in three categories of syllables and element versatility. Both approaches confirmed our hypothesis, with birds singing both more variable syllables and seemingly changing their elements more often than the structurally regular songs recorded in the breeding populations. These results are supporting the hypothesis of a role for winter-singing in song improvement, with a potentially delayed functional value during the next breeding season.