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Kodowanie informacji o gatunku i osobniku w śpiewie afrykańskich turkaweczek (*Turtur* sp.)

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Coding species and individual information in the song of African wood doves (*Turtur* sp.)

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LIST OF ORIGINAL PUBLICATIONS

My doctoral dissertation consists of (i) 2 published papers:

Niśkiewicz M, Szymański P, Budka M, Osiejuk TS (2023) Response of forest *Turtur* doves to conspecific and congeneric songs in sympatry and allopatry. *Scientific Reports* 13, 15948. doi: 10.1038/s41598-023-43035-8

Niśkiewicz M, Szymański P, Zampa L, Budka M, Osiejuk TS. (2024) Neighbour-stranger discrimination in an African wood dove inhabiting equatorial rainforest. *Scientific Reports* 14, 4252. doi: 10.1038/s41598-024-53867-7

(ii) 1 manuscript in revision:

Niśkiewicz M, Szymański P, Zampa L, Budka M, Osiejuk TS (in revision) Response of the emerald-spotted wood-dove to the song of conspecific males and sympatric congeners. Submitted to *Ethology* ETH-23-0167

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List of remaining papers I co-authored:

Zawierucha, K., Buda, J., Azzoni, R. S., **Niśkiewicz, M**., Franzetti, A., & Ambrosini, R. (2019) Water bears dominated cryoconite hole ecosystems: densities, habitat preferences and physiological adaptations of Tardigrada on an alpine glacier. *Aquatic Ecology* 53, 543-556. doi: 10.1007/s10452-019-09707-2

Łukasiewicz, A., **Niśkiewicz, M.**, & Radwan, J. (2020) Sexually selected male weapon is associated with lower inbreeding load but higher sex load in the bulb mite. *Evolution* 74, 1851-1855. doi: 10.1093/evolut/qpad139

Szymański, P., **Niśkiewicz, M.**, Budka, M., Zampa, L., Osiejuk, T. S., Skoracki, M. (2023) Quill mites of the family Syringophilidae (Acariformes: Prostigmata) parasitising doves of the genus Turtur (Columbiformes: Columbidae). *Systematic and Applied Acarology* 28(9), 1466-1475. doi: 10.11158/saa.28.9.2

Łukasiewicz, A., Porwal, N., **Niśkiewicz, M.**, Parett, J.M., Radwan, J. (2024). Sexually selected male weapon increases the risk of population extinction under environmental change: an experimental evidence. *Evolution* 77, 2291-2300. doi: 10.1093/evolut/qpad139

Szymański, P., **Niśkiewicz, M.**, Budka, M., , Osiejuk, T. S. (under review) Year-round vocal activity of the Tambourine Dove *Turtur tympanistria* in a seasonal Afrotropical mountain environment. Submitted to *Tropical Ecology* TRO-S-23-00282

SUMMARIES

Streszczenie

Śpiew i wokalizacje ptaków pełnią istotną rolę w ich codziennym życiu, ale szczególnie ważne są w kontekście znalezienia partnera i obrony terytorium. Żeby pełnić te funkcje, śpiew powinien zawierać informację o ich przynależności gatunkowej, a jednocześnie zawierać cechy umożliwiające rozpoznawanie osobników wewnątrz gatunku. W mojej rozprawie badałam afrykańskie turkaweczki, gołębie z rodzaju *Turtur*, opisując po raz pierwszy ich repertuar wokalny, głównie pod kątem rozpoznawania gatunkowego i osobniczego, a także przeprowadzając eksperymenty z playbackiem, testujące wybrane funkcje śpiewu. W tym celu brałam udział w badaniach terenowych w Nigerii, Mozambiku, Ghanie i Ugandzie w latach 2019–2023 zbierając dane dotyczące śpiewu i innych aspektów biologii. Ponieważ ten rodzaj nie był dobrze poznany, początkowo zbieraliśmy podstawowe dane biometrycznych, próbki krwi oraz piór, a także obserwowaliśmy zachowania i nagrywaliśmy wokalizacje. Dane te pozwoliły na poznanie powiązań filogenetycznych pomiędzy badanymi gatunkami, ich cech morfologicznych, preferencji środowiskowych a przede wszystkim na pełny opis zmienności śpiewu. Analizy genetyczne potwierdziły zakładane związki filogenetyczne między gołębiami, w których dwa większe gatunki preferujące leśne habitaty są ze sobą bliżej spokrewnione niż z pozostałymi trzema gatunkami z otwartych terenów, co pokazywały również dane morfologiczne. Analiza śpiewu wykazała dwuczęściową strukturę piosenek, gdzie u wszystkich gatunków początkowa część jest bardziej różnorodna, cichsza i zawiera duży potencjał do kodowania informacji specyficznej osobniczo. Z kolei końcowa część jest bardziej jednorodna, głośniejsza, przez co niesie się na dalsze odległości, mając większy potencjał do kodowania informacji o gatunku. W drugiej fazie badań przeprowadzaliśmy eksperymenty z playbackiem w celu sprawdzenia naszych założeń teoretycznych dotyczących rozpoznawania gatunkowego i osobniczego. W pierwszym eksperymencie testowaliśmy dwa leśne gatunki (*T. brehmeri* i *T. tympanistria*) żyjące w populacji sympatrycznej oraz jednego z nich (*T. tympanistria*) w populacji allopatrycznej. Ptakom zaprezentowano playbacki ze śpiewem ich własnego gatunku, gatunku blisko spokrewnionego oraz kontroli. W populacji sympatrycznej oba badane gatunki reagowały silnie tylko na śpiew własnego gatunku, a w allopatrycznej samce *T. tympanistria* reagowały na playback obu gatunków gołębi. Podobny eksperyment przeprowadziliśmy na dwóch gatunkach sawannowych (*T. afer* i *T. chalcospilos*) żyjących w populacji sympatrycznej z dodatkowym, mieszanym playbackiem zawierającym pierwszą część piosenki jednego gatunku z końcową drugiego i odwrotnie. Badane samce (*T. chalcospilos*) reagowały silnie tylko na własny gatunek, zupełnie jak w przypadku gołębi leśnych. Natomiast playback mieszanych śpiewów nie wywoływał mocnej reakcji, co świadczy o tym, że jedna część piosenki nie wystarcza by ptak rozpoznał ją jednoznacznie jako należącą do swojego gatunku. Ostatni eksperyment dotyka już problemu rozpoznawania osobniczego i został przeprowadzony *T. brehmeri*. Samcom zostały zaprezentowane playbacki sąsiadów oraz obcych ptaków z lokalnej populacji. Gołębie reagowały dużo silniej na playback obcego niż sąsiada, co pokazuje, że są w stanie rozpoznawać się indywidualnie i reagują zgodnie z hipotezą drogiego wroga. Podsumowując, turkaweczki tworzą grupę z podobną składnią śpiewu, jednak zawsze specyficzną gatunkowo i charakterystyką ukształtowaną zgodnie z preferencjami środowiskowymi i jego akustyką. Zbadane eksperymentalnie gatunki wykazują zachowania wskazujące, iż podczas interakcji terytorialnych są one zdolne do przetwarzania informacji związanych z kodowaniem tożsamości na różnych poziomach, co pomaga im w podejmowaniu optymalnych decyzji dotyczących obrony terytorium i wabienia partnerki.

Słowa kluczowe: wokalizacje, składnia śpiewu, rozpoznawanie gatunkowe, rozpoznawanie osobnicze, eksperymenty z playbackiem, *Turtur* sp.

Abstract

The song and other vocalisations of birds play an important role in many aspects of their daily lives but are particularly important in the context of mate attraction and territory defence. To fulfil these functions, song should enable birds to convey information about species identity and, at the same time, contain features that allow individual discrimination within a species. In my thesis, I have studied African doves of the *Turtur* genus, exploring their vocal repertoire, mainly in terms of species and individual recognition. To this purpose, I participated in field research in Nigeria, Mozambique, Ghana and Uganda between 2019 and 2023, collecting data on the vocalisations and other biological features of the *Turtur* doves. As this group of birds was not well studied, the first trips were mainly dedicated to collecting biometric data, blood and feather samples, as well as, observing the behaviour and recording the vocalisations of individuals from each species. These data allowed us to describe the phylogenetic relationships between the species, their morphological characteristics and habitat preferences, and – most of all - a complete description of within and- between species song variation. The analysis of the genetic material confirmed the assumed phylogenetic relationships between doves, in which two larger species preferring a forest habitat are more closely related to each other than to the remaining three species from more open areas, as also shown by the morphological data. Song analysis of all species showed shared two-part song structure, where in all species, the initial part is more diverse, quieter and has a high potential for encoding individual-specific information. Conversely, the final part is more homogeneous in structure and louder across the genus, and carries over further distances, having more significant potential to encode information about the species. During the study's second phase, we conducted playback experiments to test some theoretical assumptions about species and individual recognition in the *Turtur* genus. In the first, we tested two forest species (*T. brehmeri* and *T. tympanistria*) living in sympatry and one of them (*T. tympanistria*) in an allopatric population. Both groups were introduced to playbacks with the song of a conspecific, a congeneric and a control. In the sympatric population, both tested species responded strongly only to the songs of their own species, whereas in the allopatric population, *T. tympanistria* males responded to the playback of both dove species. We conducted a similar experiment in two savannah species (*T. afer* and *T. chalcospilos*) living in sympatry with an additional playback containing the first part of the song from one species with the final part from the other, and vice versa. The tested males (*T. chalcospilos*) reacted strongly only to their own species, like forest doves. In contrast, the additional mixed playback did not elicit strong responses, indicating that one part of the song is insufficient for the bird to categorised the song as coming from its species. The last experiment addressed the problem of individual recognition and was conducted on the largest forest species (*T. brehmeri*). The males were presented with playbacks of songs from their neighbour and stranger birds of their species from the same population. Doves reacted stronger to the playback of strangers than neighbours, indicating – for the first time for doves - that also in this family, birds are able to discriminate between neighbours and strangers and behave according to the dear enemy hypothesis predictions. Summarising the results from the study presented in this thesis, wood doves of the genus *Turtur* share a song of a similar, two-part structure but with both species- and individually specific phrases, and they are capable of processing this identity information during territorial interactions, which helps them to make optimal decisions regarding territory defence and mate attraction.

Keywords: vocalisations, song syntax, species recognition, individual recognition, playback experiments, *Turtur* sp.

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NARODOWE CENTRUM NAUKI

GENERAL INTRODUCTION

Animal communication

Animals constantly perceive and process information from their environment, especially in the context of sounds, which come from both other animals (including the own species) and sounds from the environment (*sensu* geophonies and anthropophonies; (Farina, 2014)). Animals can gain a lot of information which is crucial for their survival and reproduction, as well as to adapt their behaviour to the acoustic environment, e.g. natural or anthropogenic noise (Girola et al., 2023; Nemeth and Brumm, 2010). However, background sounds are not as important as vocal messages from other animals, since communication underpins most animal behaviour (Bradbury and Vehrencamp, 2011). Animals can discern impending threats conveyed through alarm calls by simply eavesdropping on conspecific vocalizations without actively participating in communication (Ey and Fischer, 2009; Semple and Higham, 2013), or use eavesdropped signals to locate the source of the food (Clay et al., 2012). However, from the perspective of reproductive success, more important is intentional communication between animals of the same species, which is necessary for attracting a mate, territory defence or feeding offspring (Catchpole and Slater, 2008; Collins, 2004; Godfray, 1991).

The importance of song in birds

Birds are one of the most vocal animal taxa. They produce vocalisations typically divided into songs and calls categories; however, this division is not always clear and straightforward. Some researchers limit the song term only to songbirds (Oscines), however, in my opinion it is better to define songs as species-specific, relatively complex and loud vocalisation produced during breeding season (Catchpole and Slater, 2008; ten Cate et al., 2002). Song of birds is incredibly diverse, from simple notes produced in a repetitive manner, like do some doves, to long, continuous vocal display containg hundreds of different syllables with very diversified structure, like skylarks (Briefer et al., 2008; Fletcher et al., 2004). Regardless of the level of complexity, songs fulfil two primary functions: mate attraction and rival deterrence (Podos et al., 2004). The advantage of songs as a signal is that receivers can hear them from a distance, even if a sender is far away and not visible due to obstacles in habitat (e.g. trees or shrubs). To fulfils both mentioned functions, song has to be enough species specific that both potential mates and competitors can correctly classify it as produces by conspecific individual. At the same time, the song must be sufficiently variable within species that the partner or rival will be able to discriminate between different individual classes (e.g. stranger vs neighbours) or even recognise particular individuals (Tibbetts and Dale, 2007). These two levels of encoding information in songs have evolved in parallel with the birds' ability to decode it with sufficient certainty. This evolution was affected by many factors and limitations, from phylogenetic constraints to acoustics of habitats to acoustic competitors, to mention the most important (Farina, 2014).

Species recognition

In birds, mate recognition and courtship are predominantly regulated by vocal signals, hence, song is often the most important prezygotic isolation mechanism (Brambilla et al., 2008). For example, the eastern and western meadowlarks (*Sturnella magna* and *Sturnella neglecta*), are similar-looking members of family Icteride living in North America. While their visual differences may be subtle, their songs are distinct and enable recognising the own species and avoid misdirected mating in area where their ranges overlap (Falls 1981). Sometimes perception of song is going even further and females are able to recognise local dialects and select males based on their origin (Toews, 2017)

That is why from the females' perspective potential error in species recognition is very costly as may lead to misdirected reproduction and wasting a limited number of breeding attempts (Grant and Grant, 1996; Seddon, 2005).

And although from the perspective of the males, the situation is somewhat less dramatic, for them too, the misidentification of the species is costly. Males may engage in unnecessary conflicts or fail in territorial defence, what in further perspective may affect mating success. A well-presented, species-consistent song is also an indicator of the genetic quality of the singer, and thus preferred by females (Price, 2013; Slabbekoorn and Smith, 2002).

Individual recognition

In many birds, song of individuals belonging to the same species are different while still have all necessary species-characteristics. This level of variation enables receivers to discriminate between different individuals, however, this process is diversified and may have different functions. For example, in king penguins (*Aptenodytes patagonicus*), chicks identify the call of its parents what facilitate feeding them (Jouventin et al., 1999). This acoustic system is very sophisticated as communication take place in an acoustically constraining environment with high level of noise. An analogous system was also found in other non-nesting penguin species, ie. the emperor penguin (*Aptenodytes forsteri*), where the parents have to find each other in order to swap care for the young (Aubin et al., 2000). These examples demonstrate the extreme level of selection on some kinds of signals, however, not always signals have to be as much individual and redundant. For example, penguin species with nests, prefers finding chicks by location (Jouventin and Aubin, 2002).

The most extensively studied aspect of song recognition likely pertains to territorial behaviour. In the majority of bird species, particularly those inhabiting temperate climates, males establish and defend territories against other rivals of the same species and sex (Catchpole and Slater, 2008). Efficient defence of territory involves singing and responding to the songs of rivals while costly behaviour like patrolling, chasing or even fight are limited (Brown, 1964). The crucial aspect of this behaviour is to keep cost of defence at reasonable level. This is possible if birds can recognise individuals or at least discriminate between neighbours and strangers and prevent unnecessary conflicts with familiar individuals (Temeles, 1994). This reduction of aggression towards neighbours has been called the "dear enemy phenomenon" (Fisher, 1954), and was demonstrated in many species of songbirds as well as in several non-learners (reviews in (Budka and Osiejuk, 2013; Stoddard, 1996).

The genus *Turtur* as a representation of non-singing birds

Researchers working on birds' communication often contrast songbirds (Oscines) with their incredible sound production capabilities with other groups. Songbirds indeed can sing with astonishing variability which results from a few factors. Firstly, they possess vocal apparatus (syrinx) characterised by very precise control over the muscles and membranes allowing for fine-tuning of sound production. Their syrinx has bilateral structure and these two half can produce sound independently. Secondly, they are song learners and are mimic sound produced by other individuals, or in extreme cases, any sounds heard (Ten Cate, 2021). Most other birds also have syrinx, but it is much simpler and does not allow for such perfect control over the sound produced. However, according to learning, we have two other groups that acquire their songs through mimicking, namely hummingbirds (Trochilidae) and parrots (Psittaciformes). Of the two, only parrots seem to be vocally as capable as songbirds, however, by meaning of syrinx structure and sound production details they achieve it with different mechanisms then Oscines (Goller, 2022).

Doves, which are subject of my thesis belong to non-learners and their syrinx has rather simple structure, which is relatively well known (Elemans et al., 2008). Their songs are usually very simple in structure and often consist of a repeated single phrase. However, the family Columbidae encompass 353 species and it is possible to find species with a little bit more elaborated songs. What do they lack, in comparison to songbirds is repertoire. In a better studied species it is possible to indicate different categories of signals, for example, loud songs (or calls) for advertisement and deterrence of rival and softer courtship songs used towards mates. But these signals are not produced in dozens or hundreds of versions, within a species they seem to be very similar among different individuals.

Doves belonging to the *Turtur* genus (wood doves) encompass five species commonly observed in sub-Saharan Africa, all presently designated with a conservation status of least concern. Wood doves exhibit habitat versatility, ranging from lowland rainforests, montane forests, and different types of woodland to savannahs. Their primary dietary sources comprise grass and herb seeds, insects, larvae, and occasionally small snails. Typically, foraging occurs solitarily or in pairs on the ground, though in locales abundant with sustenance, small group foraging may ensue. While generally sedentary, certain populations display partial migration in response to alterations in food availability or climatic conditions. Breeding patterns lack a distinct seasonality. Monogamous pairs, when initiating the breeding process, construct modest nests using twigs and plant litter, typically accommodating two eggs. The incubation and subsequent brooding period spans approximately two weeks, with limited information available on nest parasites. *Turtur* doves are presumed to exhibit a lengthy lifespan, exemplified by *Turtur chalcospilos*, which is documented to live between 8.5 and 9 years. Throughout their lifespan, males actively uphold territorial boundaries, occasionally leading to direct confrontations with other males.

The direct reason for choosing wood doves as a model to study was their characteristic songs. They do not resemble song of other doves, are long and consist of short notes forming a song phrase with characteristic syntax. Despite superficial similarity both among species as well as within particular species, they were found (in one species) to carry very precise information about male identity (Osiejuk et al., 2019). Close phylogenetic affinity, similar morphology, variation in environmental and food preferences and, most importantly, their long, heritable vocalisations with the potential to encode identity information made the *Turtur* genus an ideal study system to represent non-learning birds.

The first aim of the project was to to describe in a standardized way the song variation among and within species of all *Turtur* sp., with special focus on parallel analyses of relatedness between species, their morphology and habitat preferrences.. The material collected allowed us not only to provide a comprehensive description of wood doves song, but also to show how exactly their acoustic signals differs at species- and individual level (see Chapter 1). The second aim was to test if males of two forest wood doves (*T. brehmeri* and *T. tympanistria*) respond in territory defence context to song of conspecific and congeneric species in both the sympatric and allopatric ranges territorial (see Chapter 2). In another step we asked a similar question, but experimental tests were done on males of savannah specialist *Turtur chalcospilos*, co-occurring with *Turtur afer.* We additionally tested, in this case, if mixed song phrases build from halfs of the songs of these two species are able to evoke response of territorial males (see Chapter 3). Finally, we tested if *T. brehmeri* respond to the songs of neighbours and strangers as anticipated by the dear enemy hypothesis (see Chapter 4).

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CHAPTER 1

Inter- and intra-specific variation of song in African wood doves – effects of phylogeny, morphology, and habitat

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Unpublished manuscript

Abstract. Song and territorial calls serve for territorial defence and female attraction in birds. Vocal signals are essential in long-range communication and efficient in visually obscured habitats. However, individuals face the problem of correct discrimination between their own and other species and between neighbours and strangers of conspecifics. The wood doves (genus *Turtur*) comprise five species inhabiting diverse forest and savanna habitats in sub-Saharan Africa. Allopatric populations of a single species and locations where 2-3 species coexist are possible. All *Turtur* doves produce characteristic low-frequency and relatively long songs. These songs sound very similar to humans, but experience allows us to recognise species and individuals using computer-aided tools. For the first time, we investigated inter- and intraspecific variation in territorial calls for the entire genus. We found that song frequency ranged from 246 to 703 Hz, durations of phrases reached 24 s, and a number of note-building phrases was very variable (14-54). Songs of all wood doves shared similar basic syntax characterised by a two-part structure. The initial part of the song phrases was always more individually variable and had lower amplitude. The final part of the song phrases were louder and more species-specific. We identified several similarities and differences in such details of song as note duration, pause duration, frequency, rhythm, and finally absolute and relative amplitude. The pattern of variation we observed was related to genetic relationships between species, their morphological differentiation, and habitat preferences. Closer related forest species were larger and had lower frequency songs, with shorter, and less modulated notes. The three species preferring more open habitats were smaller, had songs higher in frequency, and notes building their song phrases were longer. However, some exceptions from general rules were also found. The smaller forest specialist (*T. tympanistria*) had songs as low in frequency as the 40% larger congeners (*T. brehmeri*). We identified territorial songs' traits that appear to be responsible for discrimination at different levels (species, individual). The significant differences in vocalizations (e.g. frequency) we found may be related both to the body size of each species and to the type of habitat preferred. In some species, the largest potential for encoding both species and individual identity appears to be in the temporal pattern of between notes intervals, however, surprisingly, individuals consistently differed also in peak frequencies of their songs. One unexpected result concerned the relative amplitude of notes within song phrases, which could be as low as -30 dBA SPL (maximally even -46 dBA SPL) compared to the vocalisation maximum. In all species, the initial part of the song phrase has a lower amplitude. This result suggests that males limit the active range of some parts of the signal they emit.

Key words: song structure, song variation, song syntax, interspecific differences, acoustic adaptation hypothesis, phylogeny, habitat preferrences, wood doves, *Turtur* sp.

1. Introduction

1.1. General introduction

Signals ad communication between animals are central to these organisms and underpin all types of activity. From foraging to reproduction to functioning in complex social systems, everywhere, the efficient production of signals and adequate response to them increases the fitness of individuals. Animal signals are extremely diverse and the information they encode is transmitted through various channels, including the acoustic channel. Sound signals are used by many groups of animals: insects, crustaceans and almost all vertebrates. One of the best studied groups in this respect are birds with their diverse vocalisations (Bradbury & Vehrencamp, 2011). The song is one of the most important signals used by birds during their daily basis activity. The term song has several definitions, here we will follow this presented by C. ten Cate and co-authors: "During the breeding season, many birds produce species-specific, relatively complex and loud vocalizations, usually referred to as "songs" (ten Cate et al., 2002). Both males and females can produce songs, but in most species, males do sing during the breeding season to attract females and deter rivals. The advantages of acoustic signals lie in their potential to cover large distances (well out of sight in many environments) at which they may act by conveying information and allowing complex social interactions (Catchpole & Slater, 2008). However, the effectiveness of such interactions depends on the ability of signal receivers to distinguish conspecifics from other species. In birds, which often find themselves far apart during interactions, acoustic signals play a crucial role in determining subsequent behaviour. Important decisions, such as approaching potential mates or preparing for an intruder attack, initially rely entirely on information encoded in the acoustic signal.

In the 1960s, Claude Bremond started extensive experimental research that focused on finding how (or where in the song) the information enabling species recognition was encoded (reviewed in (Catchpole & Slater, 2008)). His research led to a remarkable growth of similar playback experiments in various species and essential conclusions. Research has shown that species have specific song features which enable species recognition (Catchpole & Slater 2008). However, these characteristics, like syntax, element structure, timing, or frequency, differed among species. Secondly, in some species, redundancy in species identity coding was found. One of the predictions widely supported in later research was that song features used for species recognition should be the most invariable within a species (Marler, 1960). Here we come to important questions. Why do species differ in species-specific song characteristics? What kind of factors affect the evolution of species-specific song characteristics? Species that are evolutionarily closer to each other should have more similar signals, at least because of anatomical similarities that affect sound production (Podos, 1997). On the other hand, if they are similar and inhabit similar habitats, the potential for interspecific interaction is likely the highest. Consequently, one should observe strong selection pressure on tuning signals in a way that enables efficient species recognition despite the overall similarity.

Several hypotheses were raised to answer the mentioned above questions. As a song acts only if it reaches the receiver, environmental factors which can influence song structure due to differences in the sound propagation through different habitats (acoustic adaptation hypothesis, later AAH) were suspected to affect song evolution seriously (Morton, 1975). The AAH predicts that signals propagating through forest environment should have lower frequency and longer pauses between notes than those produced in open habitats due to scattering by refraction, reflections and diffraction from vegetation (Wiley, 1991; Wiley & Richards, 1978). Another hypothesis proposed to explain between-species song variation is the morphological adaptation hypothesis (later MAH), suggesting that body size and beak morphology, in particular, should affect the sound frequency and temporal pattern of sound production (Podos, 2001). Simultaneously, morphology is strongly affected by phylogeny and many ecological factors, such as type of diet and foraging (Grant & Grant, 2006). The picture of song evolution becomes even more complicated if we enrich the model with the sensory component as the receiver sensory detection properties, such as sensory system, brain characteristics, etc., interact with the features of the physical environment (Cummings & Endler, 2018; Yorzinski, 2017). Moreover, if congeners occur in the same environment, conspecific recognition may become another important driver of divergence in signals necessary for mating (Schoen et al., 2023). The acoustic niche hypothesis (later ANH) states that the sound spectrum is a limited resource, and species must reduce acoustic competition (Farina, 2014; Krause, 1993). A main prediction of this hypothesis is a partitioning of frequencies used by species sharing a particular environment, and it was found in bats, birds, and cicadas in the (Farina, 2014). Finally, the species recognition hypothesis (later SRH) predicts that species living in sympatry try to reduce the risk of utilising similar acoustic signals that could confound species in reproduction and increase the risk of hybridisation. SRH was confirmed in neotropical suboscine passerines, in which the song of sympatric pairs of closely related species is more different than that of allopatric pairs (Seddon, 2005).

Birds with their song as a model group for studying species recognition have an additional advantage. Among birds, we have both groups where singing is based on learning (Oscines, parrots, hummingbirds) and those where song production is inherited (Catchpole & Slater, 2008). As changes in behaviours based purely on the genetic component compared to those where both genes and social learning by copying behaviours of other individuals may have different tempos and patterns, again, we may expect differentiated evolutionary pathways shaping the species recognition process in birds. Most of the research on this topic was conducted on songbirds. However, having usually simpler songs, non-learners among birds seem to have no problem fulfilling all Pole's essential song functions(Schoen et al., 2023).

Species recognition is only a prerequisite for the song to modify the receiver's behaviour. In several species, it has also been shown that a song may have a so-called local signature, typically a phrase or sequence shared by all members of a local group within a given area. There are many categories of such variation, differing in sharing pattern, area covered etc. (reviewed in (Catchpole & Slater, 2008). Furthermore, individual recognition or at least the possibility to discriminate between some classes of individuals (e.g., neighbour vs stranger, or own mate vs intruder male, offspring–parent) is no less critical than species recognition (Budka & Osiejuk, 2013; Carlson et al., 2020; Jouventin et al., 1999). It is just the next step, and this level of discrimination or even recognition is essential for territorial defence, mating, and breeding success. Although many studies addressed this coding aspect, most research addresses whether individuals can recognise individuals or discriminate between signal classes; fewer studies showed how exactly the identity information is encoded and proved this experimentally (Carlson et al., 2020).

1.2. Short introduction to study model

African wood doves from the genus *Turtur* are small and evolutionary distant (to other doves) taxa (Khan & Arif, 2013; Lapiedra et al., 2013) characterised by song features, making them a great model for studying the evolutionary trade-off between coding species recognition and individual recognition within close relatives. The genus contains five species: the blue-headed wood-dove (*Turtur brehmeri*), the tambourine dove (*Turtur tympanistria*), the blue-spotted wood-dove (*Turtur afer*), the emerald-spotted wood-dove (*Turtur chalcospilos*), and the black-billed wood-dove (*Turtur abyssinicus*) (see Supplement 1 for photos of all species). Firstly, all five species within this group produce similar vocalisations, regardless of whether we evaluate this aurally or measure acoustic parameters directly. Secondly, all species live in a range of forest (*T. brehmeri* and *T. tympanistria*), woodland and savanna-like habitats (*T. afer*, *T. chalcospilos*, *T. abyssinicus*) in Sub-saharan Africa. They may occur both in allopatry and in sympatry where two or three species could be found together, suggesting that they may potentially be exposed to the vocalisations of other *Turtur* species and may compete for the same resources (Baptista, Trail, Horblit, & Boesman, 2020b, 2020c, 2020a; Baptista, Trail, Horblit, Boesman, et al., 2020a; Kirwan et al., 2021). Therefore, asking how they recognise their species and if and how they recognise or discriminate between individuals is vital for understanding the evolution of signalling in this group. Indeed, a recent study showed that coexistence, or lack, between two forest dove species resulted in differentiated responses to the playback of the own species and songs of the congeners (Niśkiewicz, Szymański, Budka, et al., 2023).

1.3. Aims of the study

This study forms the descriptive component of the project, which aims to elucidate the mechanisms enabling species and individual recognition within a group of *Turtur* doves. In principle, it was aimed at providing a theoretical background for the experimental part of the project, allowing the formulation of specific hypotheses. Specifically, this study aimed to describe in detail the song structure of all African wood doves, emphasising song characteristics that have the largest potential for coding species specificity and those that are likely crucial for coding identity of males.

Firstly, we hypothesised that species-specific information should be encoded in the part of song, which propagates at larger distances. Therefore, one would expect it to be a louder and/or lower frequency part. Recognising one's species is essential both for rivals and females, and maximising this distance seems to be the best strategy for each individual. On the other hand, individual recognition or discrimination between a smaller number of individuals (e.g., between neighbours and strangers, or groups of neighbours and intruders, etc.) could be a priori limited to a shorter range (Aubin et al., 2014). It is not necessary to recognise or discriminate between hundreds of individuals, but it is crucial to be able to discriminate between closest neighbours and non-neighbours (Temeles, 1994). Moreover, limiting the possibility of recognising individuals at a distance could sometimes be advantageous according to the deceptive mimicry hypothesis (Wilson & Vehrencamp, 2001). Therefore, we compared in this study how fragments of songs characterized by different frequencies and relative (within song-phrase amplitude) encode species and individual identity. We hypothesised that songs as a long-distance signal should have encoded information about species and individual identity. We assume that identity could be encoded in such parameters as the frequency of notes, duration, and detailed characteristics of sequences built by the note and between note pauses, as their large number within song phrase creates a considerable potential for forming species and individually specific signals (Osiejuk et al., 2019). We also suppose that coding identity characteristics may be affected by the habitat of living species and the fundamental differences in their body characteristics, like size. Finally, as the *Turtur* doves are relatively little studied and there is a lack of essential information in the literature, we present data on their phylogenetical relationships, morphology and habitat preferences, which could be a background for the discussion on song evolution in this group.

3. Methods

3.1. Study areas

Data used in this paper were collected between 2008 and 2023 in several African countries: Cameroon, Nigeria, Mozambique, the Republic of South Africa, Ghana, and Uganda. However, the core of the data analyzed (Figure 1) was obtained between 2019 and 2023 in Nigeria, Mozambique, Ghana, and Uganda. In Nigeria, data were collected in Obudu Plateau (Obudu Cattle Ranch; N 6.37°, E 9.38°) in November-December 2019. In Mozambique, data were collected in Gorongosa National Park (S 18.97°, E 34.35°) in March 2018, May 2019, and February-March 2020. In Ghana, data were collected in Kakum National Park (N 5.35°, W 1.38°; January-February 2021, November-December 2021, and November-December 2022) and in Mole National Park (N 9.26°, W 1.86°; December 2021).

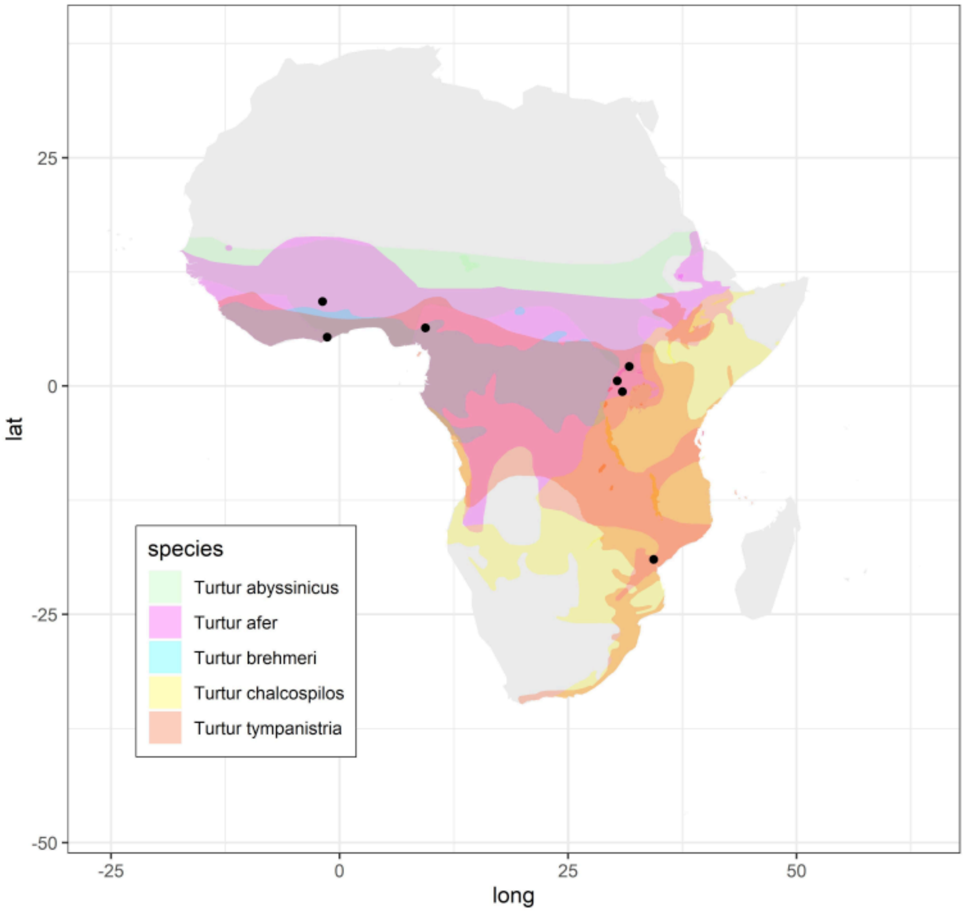


Figure 1. Distribution map of the black-billed wood-dove (*Turtur abyssinicus*), the blue-spotted wood-dove (*T. afer*), the blue-headed wood-dove (*T. brehmeri*), the emerald-spotted wood-dove (*T. chalcospilos*) and the tambourine dove (*T. tympanistria*). The locations where the data was collected are shown (from left to right, and from up to bottom): Mole National Park and Kakum NP in Ghana, Obudu Plateau in Nigeria, Murchinson Falls NP, Kibale NP and Lake Mburo NP in Uganda, and Gorongosa NP in Mozambique.

In Uganda, we collected data in Kibale NP (N 0.43°, E 30.40°; June-July 2019, 2021-202 , Murchison Falls NP (N 2.27°, E 31.68°) in July 2019, and in Lake Mburo NP (S 0.63°, E 30.96°) in July 2019, June 2022, and June-July 2023.

3.2. Fieldwork procedures

Focal birds were recorded with directional microphones, and soundscapes were recorded with autonomous recorders (ARU) positioned near observed birds. GPS information was assigned to all collected data regarding time and location. Some of the captured birds were tagged with LifeTags^{TM} (Cellular Tracking Technologies, USA), and over the subsequent days, they were tracked by two researchers. One person recorded the male (technical details below), while the second confirmed males' identity using telemetry (by radio signal from LifeTags). This data was used solely to confirm that the structure of an individual's song remains stable over time, making it reasonable to use for recognising individuals based on their songs.

Caught birds were measured by one person (PS). The wing length (maximum chord) and tail length were measured with a metal ruler with an accuracy of 1 mm. The tarsus length, bill length, bill height, and total head with bill length were measured with callipers to the nearest 0.1 mm. Birds were also weighed using a Pesola spring balance with an accuracy of 0.5 g and blood samples were taken for subsequent DNA analysis (described separately).

3.3. Recording equipment

Recordings of observed individuals were made with a Sound Devices MixPre-3, a Marantz PMD 661 MKII, or Sony PCM-D100 recorder, and a Sennheiser ME 67/K6 (frequency response 40 Hz-20 kHz) or Sennheiser MKH-70 directional microphone (frequency response 50 Hz-20 kHz). All equipment is characterised by superior quality, and differences between them could be neglected. Birds were recorded with 48 kHz sampling and 16 or 24 bit quality. We used several types of ARUs from Wildlife Acoustics (USA): Song Meter SM3, Song Meter SM4, and Song Meter Micro, and recordings were done with 48 kHz sampling and 16-bit quality.

3.4. Recording procedures

Singing males were recorded from the closest distance possible, and these distances varied significantly between species and habitats. For forest species, approaching a male closer to 20-30 m was difficult, while it was sometimes possible for savannah species to come closer than 10 m. The recordist attempted to record as many as possible (at least 10) songs) after reaching the optimal distance to a bird. Recording *Turtur* doves presented a challenge due to their songs' relatively low amplitude, low frequency, and long duration. The low amplitude necessitated being close to the bird for a good recording, and the low frequency made recordings more susceptible to disturbance from low, noisy sounds, such as crackling when holding the microphone. Additionally, the length of the songs, combined with low amplitude, increased the likelihood of other background sounds appearing within a focal male's song. In such cases, the entire song was excluded from the analyses.

In addition, attempts were made to collect basic measurements of the natural song amplitude, which were essential for designing future experiments and exploring the functions of *Turtur* dove songs overall. When a singing individual was within close proximity (typically 6 – 25 m, determined with a Leica DISTO D510 laser distance measurer) and visible to the observer, we used a CHY 650 decibel meter set to A/fast settings to measure the song's amplitude., Measurements were then recalculated to be presented in a standard way, i.e., dBA SPL level at 1 m from the bird.

We also estimated the distance at which the human ear could detect a singing male. Such estimations were done opportunistically when a precisely located male was recorded, and the observer was moving away from him while still noticing songs and distance (with GPS). In a few cases, such a procedure was repeated, i.e., the observer first moved away and then moved closer again to assess whether the same individual was still heard.

3.5. Bioacoustics analysis

The bioacoustics analyses in this paper were based on several attempts using different techniques and increased during the project's experience with the studied species, including those published earlier (Niśkiewicz, Szymański, Budka, et al., 2023; Osiejuk et al., 2019). The nature of how the studied birds sing caused non-trivial problems, which we explain in detail below. The main problem we discovered during the analysis was a significant difference in the amplitude of notes within the song. This natural feature of the studied species' songs (especially those from savannah habitats) forced us to work with a limited number of recordings (and individuals) because, in extreme cases, to obtain a reliable recording of an individual, the distance between a bird and recordists had to be extremely small (~ 10 m or less). Otherwise, recording all notes within the song with quality allowing for measurements was impossible. As one of the aims was to compare between- and within-individual variation of several song parameters, we limited the sample size to those individuals for whom we had high-quality recordings, for which we were able to measure parameters of all notes, some of which were over 30 dB softer than the loudest part of the song. The selection of notes (see below) was based exclusively on the quality of the recordings.

3.5.1. The procedure of bioacoustics measurements and measurement list

Recordings were visually inspected in the spectrogram window of Raven Pro software (Figure 2) and bandpass filtered (100–1000 Hz). For measurements, only those songs were selected where all the notes were visible and not overlapped by other sounds. The complete songs were used for measuring song duration, minimum and maximum frequency, and counting the number of notes in a song phrase. These measurements were taken in Avisoft SASLab Pro 5.2 in the spectrogram or spectrum window (Figure 3).

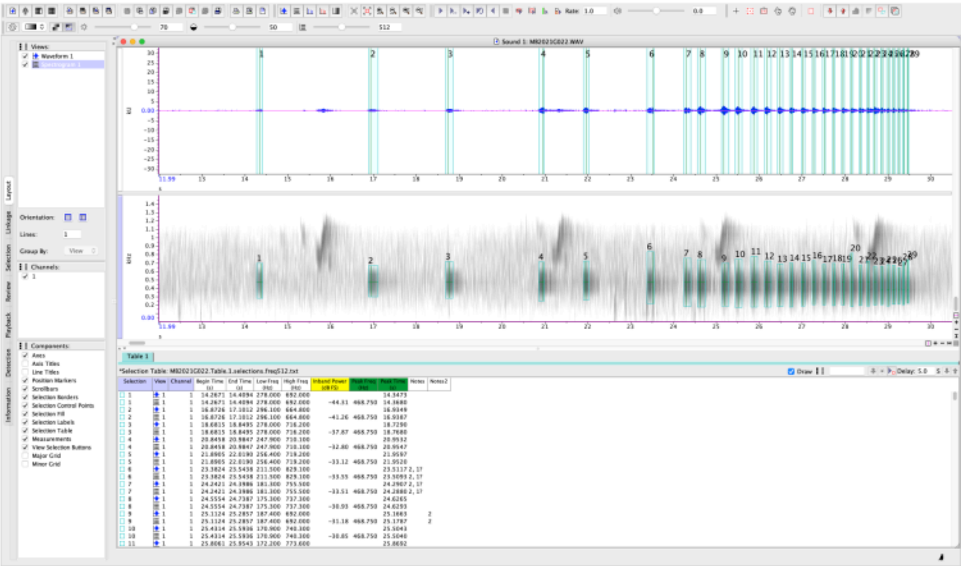
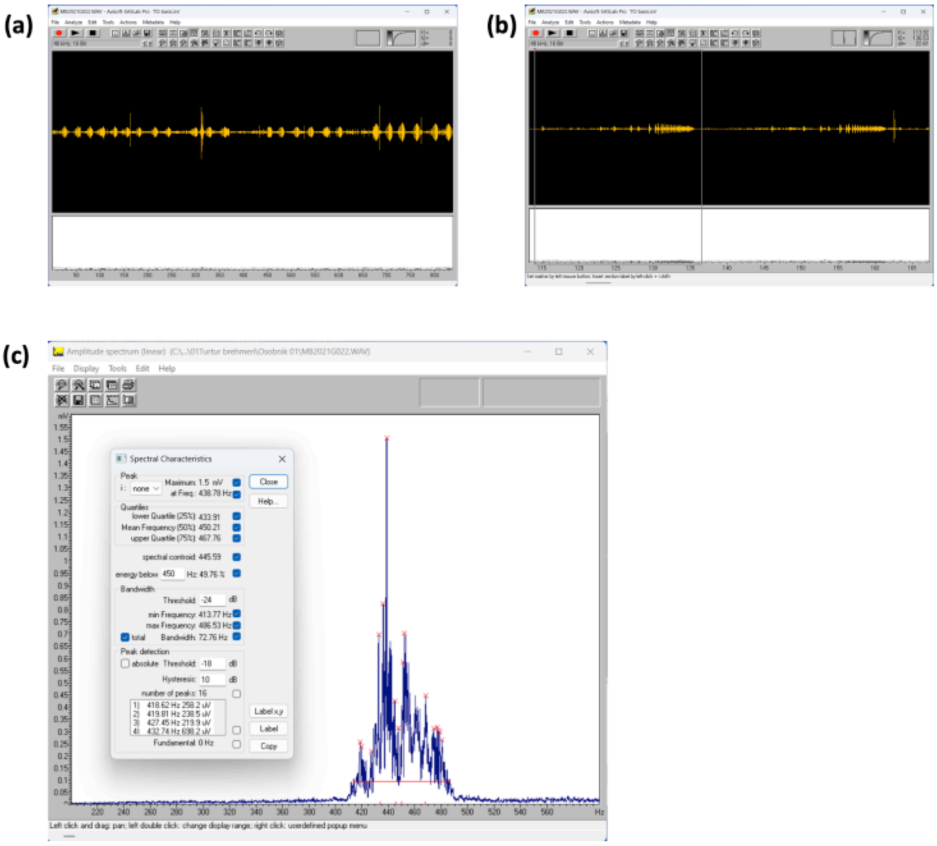


Table 1

*Selection Table: MB2021G022.Table.1.selections.freq512.txt

Selection	View	Channel	Begin Time (s)	End Time (s)	Low Freq (Hz)	High Freq (Hz)	Inband Power (dB FS)	Peak Freq (Hz)	Peak Time (s)	Notes	Notes2
1	1	1	14.2671	14.4094	278.000	692.000			14.3473		
1	1	1	14.2671	14.4094	278.000	692.000	-44.31	468.750	14.3680		
2	1	1	16.8726	17.1012	296.100	664.800			16.9349		
2	1	1	16.8726	17.1012	296.100	664.800	-41.26	468.750	16.9387		
3	1	1	18.6815	18.8495	278.000	716.200			18.7290		
3	1	1	18.6815	18.8495	278.000	716.200	-37.87	468.750	18.7680		
4	1	1	20.8458	20.9847	247.900	710.100			20.9532		
4	1	1	20.8458	20.9847	247.900	710.100	-32.80	468.750	20.9547		
5	1	1	21.8905	22.0190	256.400	719.200			21.9597		
5	1	1	21.8905	22.0190	256.400	719.200	-33.12	468.750	21.9520		
6	1	1	23.3824	23.5438	211.300	829.100			23.5117	2, 17	
6	1	1	23.3824	23.5438	211.300	829.100	-33.35	468.750	23.5093	2, 17	
7	1	1	24.2421	24.3986	181.300	755.500			24.2907	2, 17	
7	1	1	24.2421	24.3986	181.300	755.500	-33.51	468.750	24.2880	2, 17	
8	1	1	24.5554	24.7387	175.300	737.300			24.6265		
8	1	1	24.5554	24.7387	175.300	737.300	-30.93	468.750	24.6293		
9	1	1	25.1124	25.2857	187.400	692.000			25.1663		2
9	1	1	25.1124	25.2857	187.400	692.000	-31.18	468.750	25.1787		2
10	1	1	25.4314	25.5936	170.900	740.300			25.5043		
10	1	1	25.4314	25.5936	170.900	740.300	-30.85	468.750	25.5040		
11	1	1	25.8061	25.9543	172.200	773.600			25.8692		

Figure 2. The Raven Pro program window illustrates part of the workflow based on notes selection within song phrase (here, the blue-headed wood-dove). Measurements taken automatically for selections are visible graphically and in table section.



Spectral Characteristics

Peak
i: none
Maximum: 1.5 mV ☑
at Freq.: 438.78 Hz ☑

Quartiles
lower Quartile (25%): 433.91 ☑
Mean Frequency (50%): 450.21 ☑
upper Quartile (75%): 467.76 ☑

spectral centroid: 445.59 ☑

energy below 450 Hz: 49.76 % ☑

Bandwidth
Threshold: -24 dB
min Frequency: 413.77 Hz ☑
max Frequency: 486.53 Hz ☑
☑ total Bandwidth: 72.76 Hz ☑

Peak detection
☐ absolute Threshold: -10 dB
Hysteresis: 10 dB
number of peaks: 16 ☐
1) 418.62 Hz 258.2 uV
2) 419.81 Hz 238.5 uV
3) 427.45 Hz 219.9 uV
4) 432.74 Hz 698.2 uV
☐
Fundamental: 0 Hz ☐

Left click and drag: pan; left double click: change display range; right click: userdefined popup menu

Figure 3. The Avisoft SASLab Pro software windows illustrate part of the workflow based on the entire song phrase (here, the blue-headed wood-dove). Window (a) shows several songs after filtering, (b) window shows view on a single song phrase selected, and window (c) shows amplitude spectrum window, which enable taking frequency measurements with high precision.

In the second step, each note within the song was manually selected in Raven Pro by one person (MN) and checked by the second person (TSO). Selections were made for spectrograms generated with the following parameters: Window type: Hann, 1024 samples; 3 dB Filter Bandwidth: 67.4 Hz; Time grid: overlap 50%, giving Hop size: 512 samples; Frequency Grid: DFT Size: 1024 samples, resulting in 46.9 Hz x 10.7 ms resolution measurements (Figure 4). Based on these note selections, we characterised the peak frequency of notes (Hz), duration of notes (ms), and pauses between notes (ms), as well as the frequency and temporal pattern of notes production within the song phrase: note duration (ms), pause duration, note peak frequency. Measurements were derived from selections in Raven Pro, but with settings adjusted separately for frequency and time measurements, resulting in resolutions of 23.4 Hz x 21.3 ms or 93.8 Hz x 5.33 ms, respectively (Figure 4). After selection, each note was manually assigned to the initial or final part of the song (see further explanation).

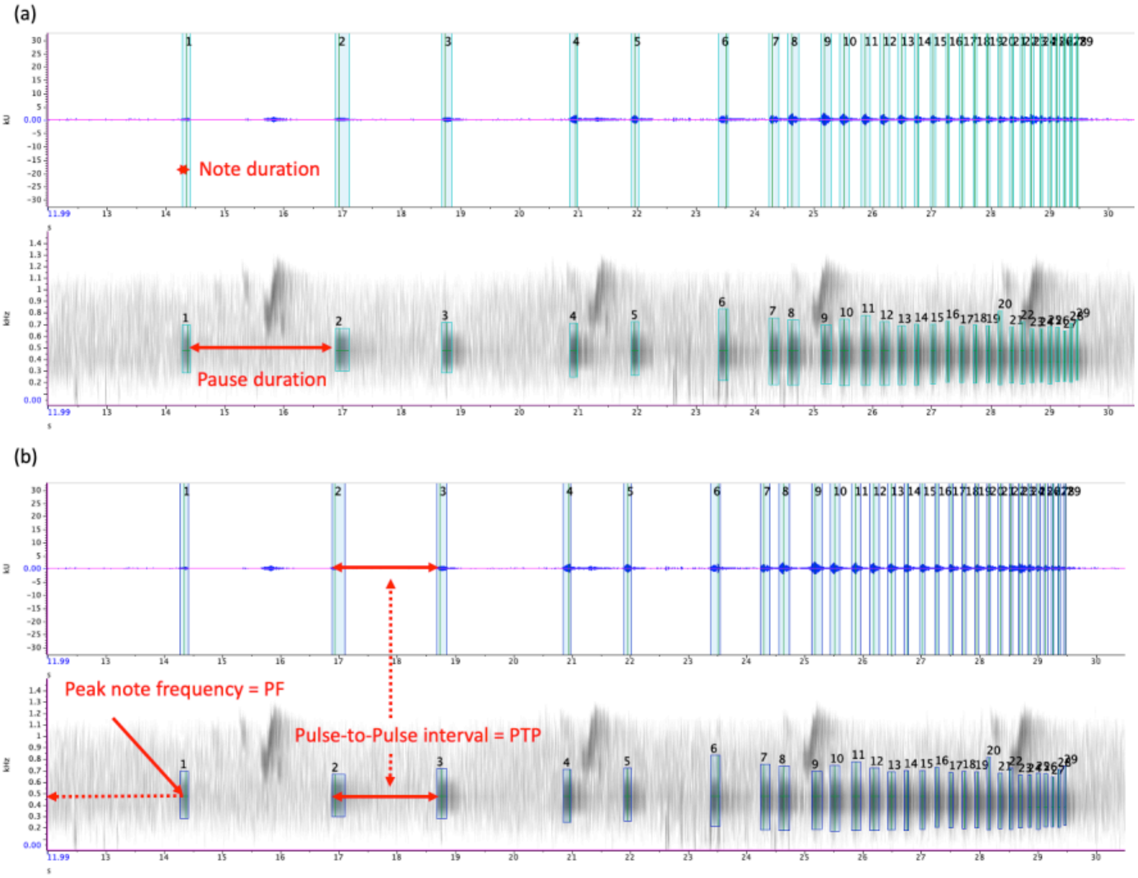


Figure 4. Windows with waveform and spectrogram of the same song phrase of the blue-headed wood-dove illustrating optimisation of time (a) and frequency (b) measurements of notes, pauses between notes, and intervals between Pulse-to-Pulse intervals. FFT settings for window (a) allow for 93.8 Hz × 5.33 ms resolutions, while for window (b), resolution is 23.4 Hz × 21.3 ms.

3.5.2. Vocabulary of terms and measurements used for *Turtur* sp. song description

Note (or element) – a continuous trace on the sound spectrogram spaced out from other traces by pause (Figure 5).

Pause – time of silence between one note's end and the start of the next within a song (Figure 5).

Song or **song phrase** – the entire sequence of notes forming repeatable vocalisation (at species and individual level); in pigeons and doves, such "song" is also commonly named calls or coo calls, but functionally they are equivalent to *Oscines* song. Song phrases could be characterised by **duration (s)** (Figure 5), **number of notes and pauses** (Figure 6), and **frequency measures (**Figure 3, Figure 4).

Note duration (s) – time from the beginning of the note to its end, measured manually based on selection in Raven Pro (Figure 4).

Pause duration (s) – time from the end of one note to the beginning of next note, measured manually based on selection in Raven Pro (Figure 4).

Note peak frequency or **PF (Hz)** – the frequency of note at which the highest amplitude was automatically found within the selection in Raven Pro. Each PF could be also characterised by precise time measurements (Figure 4).

Pulse-to-pulse interval or **PTP (s)** – time between peak frequencies of the following notes. PTP (Figure 4).

Relative amplitude or **RA (dB)** – value which could be calculated for each note within a song phrase with the formula $RA_{\text{Note } x} = PF_{max} - PF_x$, where PF_{max} is the peak frequency of the loudest note within a song phrase, and PF_x is the peak frequency of note number *x*. By definition, the RA of note with the highest peak frequency is 0.

Rhythm – the quantification of durational patterns that consist of sounds (notes) and silences (pauses), measured as the inter-onset intervals (IOIs) between two adjacent onsets of notes. This measurement enables assessing the overall distribution of IOIs within song phrases, corresponding to the rhythm structure (Figure 7) (Burchardt & Knörnschild, 2020; Sasahara et al., 2015).

Song parts, initial and final – songs of all *Turtur* doves share a two-part structure. The initial part comprises notes and pauses, exhibiting significant variability among species and individuals within a species. In contrast, the final part shows less variability, and any changes over time have a consistent direction, specifically, the shortening of note and pause durations (Figure 8).

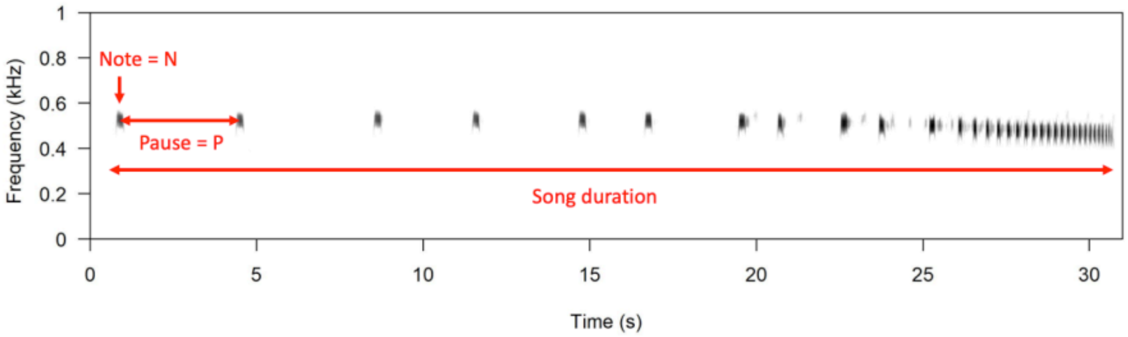


Figure 5. The spectrogram illustrates notes, pauses, and entire song duration (in *T. brehmeri*).

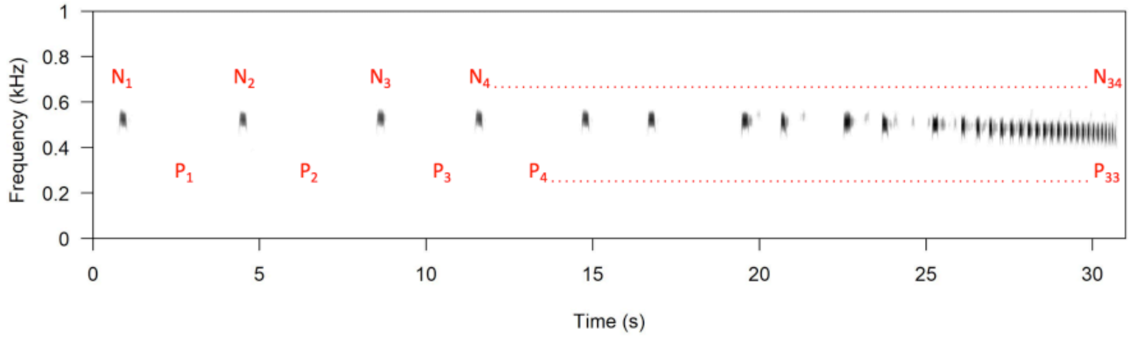


Figure 6. The spectrogram illustrates measurements taken for all notes and pauses within each song phrase. Letters indicate N_1, N_2 – durations of following notes; P_1, P_2 - pauses between following notes, based on manually selected beginning and end of each note (in *T. brehmeri*).

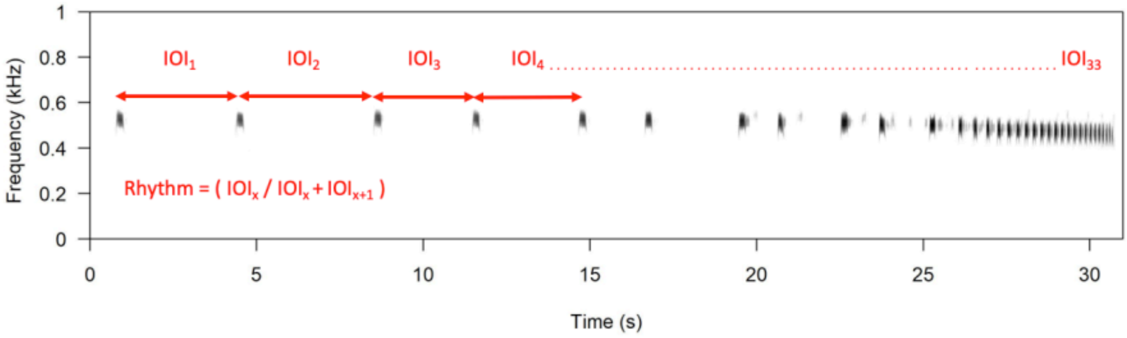


Figure 7. The spectrogram illustrates how inter-onset intervals (IOIs) and rhythm based on IOIs ratio were measured (in *T. brehmeri*).

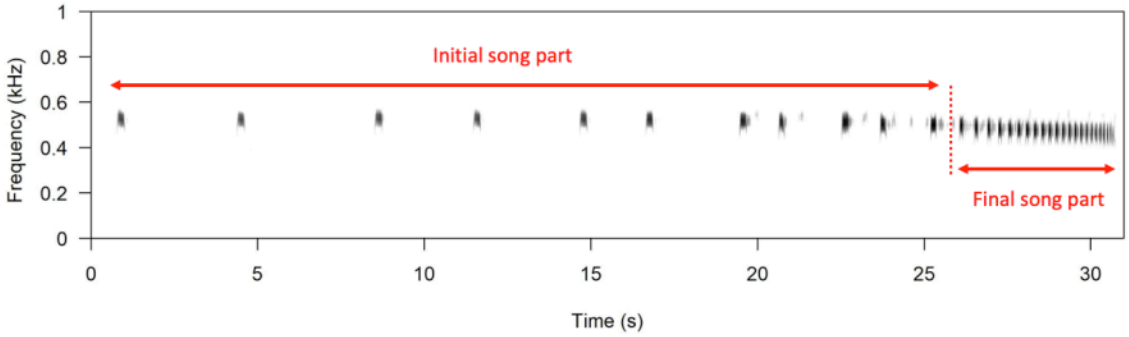


Figure 8. The spectrogram illustrates the initial and final parts of the song phrase (in *T. brehmeri*).

3.6. Species and individual identity coding

We based the analyses of potential for species- and individual-identity coding on Beecher's information statistics H_s (Beecher, 1989). H_s indicates the amount of information in a system that conveys individual identity. It has been shown to be a reliable measure of the identity potential, although as other identity measures, H_s is sensitive to sample size, either at the level of inter- or intra-individual analyses (Linhart et al., 2019). Hence, to allow direct comparisons of H_s between different variables and species, we based our analyses on 10 songs from each of 10 males of each species, i.e. 100 song phrases per species and 500 song phrases in total.

We also calculated H_s for entire song phrase measurements as well as for sequences of peak frequencies (PF) and pulse-to-pulse durations (PPT) to determine if the time-frequency pattern of notes is male-specific, as was found in the tambourine dove (Osiejuk et al., 2019). Due to variations in song duration and note numbers within and between species, we calculated H_s for sequences of 1 to 8 (counted both from the beginning and end of the song) notes consisting of PTP and PF values. This approach allows for equal (among species and individuals within species) comparison of shorter sequences, which well reflects potentially important (functionally) locations within song phrases (Niśkiewicz et al., 2024a; Osiejuk et al., 2019). All calculations were performed with the *calcHS* function of the R package `'IDmeasurer'` (Linhart et al., 2019). For comparative purposes, we also presented values of discriminant analyses (*DS*) calculated in the same package.

3.7. Evaluation of the habitat preferences of the study wood doves

We used environmental data derived from satellite imagery to assess vegetation status and to examine basic differences between habitats preferred by different wood doves. We employed the Landsat Enhanced Vegetation Index (EVI) and the Normalized Difference Vegetation Index (NDVI), widely used remote sensing metrics. These indices measure the contrast between near-infrared (reflected by vegetation) and red light (absorbed by vegetation). Additionally, EVI accounts for certain atmospheric conditions and canopy background noise, rendering it more sensitive in densely vegetated areas. Consequently, areas with healthy and robust vegetation exhibit higher absorption of visible light and greater reflection of near-infrared light. Previous studies have shown the efficacy of NDVI in determining vegetation greenness, enabling differentiation between various landscapes such as savannah, dense forests, or non-forest areas (Pettorelli et al., 2005). Both indices range between -1 and 1, with dense, healthy vegetation yielding values close to 1, 0 indicating the absence of vegetation, and negative values representing a lack of dry land.

We obtained NDVI and EVI data from the MOD13Q1 product of the Terra Moderate Resolution Imaging Spectroradiometer (MODIS) for the year 2023 (Huete et al., 2002), captured at 16-day intervals (2023-09-01 – 2023-12-27) with a spatial resolution of 250 meters. This extraction was facilitated by the `'MODISTools'` package (Hufkens, 2023). We specifically selected NDVI and EVI values corresponding to the geographical locations where we recorded territorial songs of the studied species during our research project. This was done to ensure that NDVI and EVI values represent the breeding habitat of each species. Only points separated by a minimum distance of 250 meters were considered for analysis. Additionally, we utilised data sourced from Xeno-Canto (XC), a global repository of bird recordings, focusing solely on the geographic coordinates of species-specific recordings. The retrieval of this data from the website was facilitated by the `'warbleR'` package (Araya-Salas & Smith-Vidaurre, 2017). In total, we analysed vegetation index values for 50 locations of *Turtur abyssinicus* (with 28 sourced from XC), 70 of *T. afer* (with 45 from XC), 55 of *T. brehmeri* (with 32 from XC), 184 of *T. chalcospilos* (with 103 from XC), and 175 of *T. tympanistria* (with 137 from XC).

3.7. Molecular analyses

3.7.1. Sample collection and DNA isolation

Blood samples were collected from 146 birds of both sexes from 15 populations belonging to all five *Turtur* species (Figure 1). Additionally, one male African Green Pigeon *Treron calvus* was blood-sampled and used as outgroup in phylogenetic analysis. Birds were caught with bird nest. The wing vain of the bird was punctured and outcoming blood collected directly onto FTA^{TM} Classic and Elute cards (Whatman^{TM}). The cards were dried and stored dry till the time of isolation. DNA was isolated using DNeasy Blood and Tissue Kit (Quiagen) following the protocol from manufacturer. Depending on the amount stored, two to four 2 mm pieces of card soaked with blood were cut out for DNA isolation.

3.7.2. RAD-seq library preparation and sequencing

Libraries were constructed following the 3RAD method (Bayona-Vásquez et al., 2019) and the protocol provided by its authors, with the following differences: (i) Illumina indexed primers were incorporated in a single PCR, with 12 cycles; (ii) purification at all steps was performer using AMPure XP magnetic beads (Life Sciences), following manufacturer protocol; (iii) selected size range was 230-400 bp. Samples with ligated adapters including internal indexes (i5 and i7 adapters from Table S3 in (Bayona-Vásquez et al., 2019) were mixed into two pools (combining 68 and 79 individuals, respectively) and each pool received a different combination of Illumina indexes (iTru5_001_A-iTru7_101_01 and iTru5_001_B-iTru7_101_02 form Table S3 from (Bayona-Vásquez et al., 2019), which allowed for sequencing all samples in a single lane. The paired-end sequencing (150 cycles) was performed in one lane of NovaSeq 6000 SP flow cell (Illumina), with the addition of 15% PhiX to counterbalance the effect of fixed enzyme restriction sites.

3.7.3. 3RAD processing and SNP detection

First, PhiX sequences were removed using Bowtie2 (Langmead & Salzberg, 2012) and adapters trimmed with Cutadapt (Martin, 2011). Reads were demultiplexed based on internal indexes with FLEXBAR (Dodt et al., 2012). PEAR (Zhang et al., 2014) was used to merge read pairs with overlap of at least 30 bp, while maximum length of assembled sequences was set to 229. We then discarded all assembled, i.e. less than 230 bp long, reads as out of the range of our interest, and focused on the unassembled paired-end reads. Using Trimmomatic (Bolger et al., 2014), those were filtered by quality (Q>30) and minimum length (130 bp) and cut to a maximum length of 142 bp. We used the script Check_Restricxtion_Site.py (Arantes et al., 2020) to discard any sequences digested by NheI, so that our final dataset contained only read pairs digested by XbaI-EcoRI enzyme combination. In the last pre-processing step we used Filter_Reads.py (Arantes et al., 2020) to discard any reads containing uncut restriction site of any of the three enzymes used.

SNP (single nucleotide polymorphism) calling was performed (Catchen et al., 2011). Denovo_map pipeline was used, as the data did not map sufficiently well (ca. 30%) to the reference genome of the closest species (*Columba livia*) for which whole genome data is available. Following recommendations in (Paris et al., 2017) minimum stack coverage (*-m* in *ustacks*) was set to 4, while number of mismatches allowed between sample loci in the catalog (*-n* in *cstacks*) was set to 2 and 4 for populations genetics analyses and phylogeny construction, respectively. Samples were included in the analyses based on coverage statistics from a preliminary *gstacks* run, and we retained only samples with minimum mean per-sample coverage of 10 (96 samples), while the catalog of loci (*cstacks*) was built based on 82 samples with minimum mean coverage of 30. In the *populations* program we only retained first SNP per locus (*--write-single-snp*) and set minimum frequency of minor allele (*--min-maf*) to 0.03. We calculated divergence form Hardy-Weinberg equilibrium for each locus (*--hwe*) and discarded loci departing form equilibrium at $P < 0.01$ (*-B*; n=548). Other parameters in *populations* differed depending on the scope of the analysis, so their settings are reported in the respective sections below.

3.7.4. Phylogeny reconstruction

To get data for the construction of phylogenetic tree, for *populations* (Stacks) we selected one individual with the highest coverage from each *Turtur* species and included the *Treron calvus* sample as outgroup. Only loci present in all samples were retained (*-R* 1) to avoid biased estimates due to mutations in restriction sites expected in higher frequencies between *Turtur* and outgroup than among *Turtur* species. Using this output the phylogeny was reconstructed in MEGA v.6 (Tamura et al., 2013) using neighbour-joining method with bootstrap testing (1000 replicates) and assuming Jukes-Cantor model of sequence evolution. Pairwise distances between species were estimated in MEGA using Jukes-Cantor model.

3.7.5. Population genetic analyses

For population genetic analyses, in *populations* (Stacks) the minimum percentage of individuals required to process a locus within (-r) and across (-*R*) species (i.e. 'population' in the program) were set to 0.5 and 0.8, respectively. To infer the most probable number of genetically differentiated clusters we analysed the resulting dataset (32,136 SNPs) in STRUCTURE 2.3.4 (Pritchard et al., 2000), using admixture model with uncorrelated allele frequencies and assuming the presence of null alleles. Burn-in length was set to 100,000 and post-burn-in MCMC was run for one million steps. Alpha was set to 7 to achieve good mixing. We tested values of K (the number of genetic clusters) form 1 to 15 (the number of sampled populations) and for each K value 10 runs were performed. The most probable number of clusters was inferred based on ΔK calculated in Structure Harvester (Evanno et al., 2005).

3.7. Statistical analysis

The manuscript has descriptive character and is based on many detailed measurements. To keep the results simple for, essential here, between species comparison, we used cluster analysis to present dendrograms showing summarising results for sections of analysis. These analyses mainly aimed to illustrate how similar *Turtur* doves are based on collected material concerning their biometry, habitat selection and song structure. We all used full linkage procedure with Euclidean distance from STATA 16.1 (StataCorp, Texas, USA). For the most straightforward comparisons, we also used correlation analysis and analysis of variance. For comparisons of the overall body size of individuals, the biometric data were initially subjected to principal component analysis (with SPSS Imago 9.0). In case the data analysed contained repeated measurements for individuals and/or species, we used mixed models (see below).

To test for differences between species in habitat preferences, we built Gaussian mixed model (with identity link) using the `'glmmTMB'` package (Brooks et al., 2017) implemented in R. The NDVI and EVI values were used as the response variables, and species and geographical coordinates as predictors. We also included a hemisphere (N or S) and the day of vegetation index values were collected as random intercepts in the model. Then we used predicted marginal values from mixed models to compare species habitat preferences with cluster analysis.

We tested for differences between species and part of the song (initial vs final) with generalised linear mixed-effect models (GLMM) using the `'lme4'` package of R (Bates et al., 2015). Our response variables were characteristics of notes or pauses between notes. We used the same model type for all characteristics with two main factors: (i) species (five levels) and (ii) part of the song (two levels: initial or final). We included the first-order interaction terms and used nested song identity within a male identity as a random effect.

We checked the mixed models' assumptions using the 'DHARMa' package of R (Hartig, 2020) and visual inspection of Q-Q plots. In a few cases, we transformed initial variables to meet normality assumptions. All *P* values reported are two-tailed.

3.8. Ethical note

The material used in the present study was collected during ornithological surveys in Ghana, Uganda, Mozambique, and Nigeria from 2019 to 2023. Our procedures adhered to the Association for the Study of Animal Behaviour (ASAB) and Animal Behavior Society (ABS) guidelines for the care and use of animals (ASAB/ABS 2020) and were approved by all responsible local bodies mentioned below as well as by the Polish Laboratory Animal Science Association (certificate no. 1952/2015 to TSO) conforming to Directive 2010/63/ EU. Samples were taken under local permits: (1) in Mozambique (Gorongosa National Park, Department of Scientific Services, Research and collecting permit no. PNG/DSCi/C171/2020); (2) in Nigeria (A. P. Leventis Ornithological Research Institute (APLORI), University of Jos Biological Conservatory, Laminfa, Jos East LGA, 17.09.2019); (3) in Ghana (Forestry Commission, Wildlife Division WD/A.185/Vol.13/80); and (4) in Uganda (Makerere University, College of Health Sciences; Makerere University Biological Field Station; Uganda Wildlife Authority permit no. COD/96/05, and Uganda National Council for Science and Technology permit no. NS256ES).

4. Results

4.1. Sample sizes and datasets analysed

Results presented in this study are a subset of the total material collected during the project, selected on a qualitative basis, on the one hand, and the other, based on the time capabilities of the relevant material processing. The biometry analyses were based on measurements of 155 individuals caught, of which blood samples of 146 individuals were adequate for further phylogenetic DNA analyses. As for the analysis of the audio material, we had more than 1600 recordings of focal males or point recordings (using passive acoustics monitoring PAM). In analysing between-species differences in song structure, we worked on a dataset containing 638 songs from 53 individuals. We had at least 10 song phrases per individual and at least 10 individuals per species (which gives in total 17 503 notes measured).

For estimating species-specificity and within-species individuality of songs, we used the same set of songs, but we limited the sample to strictly 10 songs per individual and 10 individuals per species, as all measures of individuality are sensitive to sample size differences between individuals (Linhart et al., 2019).

4.2. DNA analyses

4.2.1. Phylogenetic tree

On the phylogenetic tree the five *Turtur* species clustered into two well-supported lineages, composed each of species inhabiting similar environments: forest species*, T. brehmeri* and *tympanistria* cluster separately from *T. abyssinicus, T. afer* and *T. chalcospilos* inhabiting savannahs. Within savannah species *T. abyssinicus,* and *T. chalcospilos* formed another well-supported lineage. This pattern suggests earlier split between forest and savannah species and later differentiation of the lineages within each habitat.

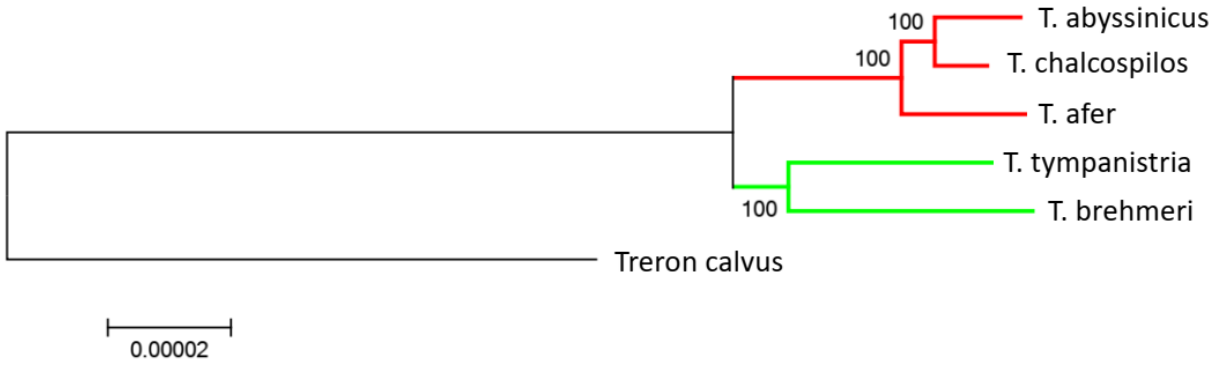


Figure 9. Neighbour-joining tree constructed from SNP data. The tree is rooted by using outgroup species *Treron calvus*. The colours of branches correspond to those from STRUCTURE plot showed in Figure 10.

4.2.2. Population/species structure

The most probably number of genetic clusters identified by STRUCTRE was two (Figure 9), with *T.* brehmeri and *T. tympanistria* in one cluster and *T. abyssinicus*, *T. afer*, and *T. chalcospilos* in another one, which seems to be in accordance with phylogenetic analysis. When number of genetic clusters was set to 5, corresponding to the number of species sampled, the clustering reflected species membership (Figure 10, Figure 11). The pattern emerging from STRUCTRUE analysis is concordant with the phylogeny, suggesting stronger genetic divergence of *Turtur* species between than within habitats.

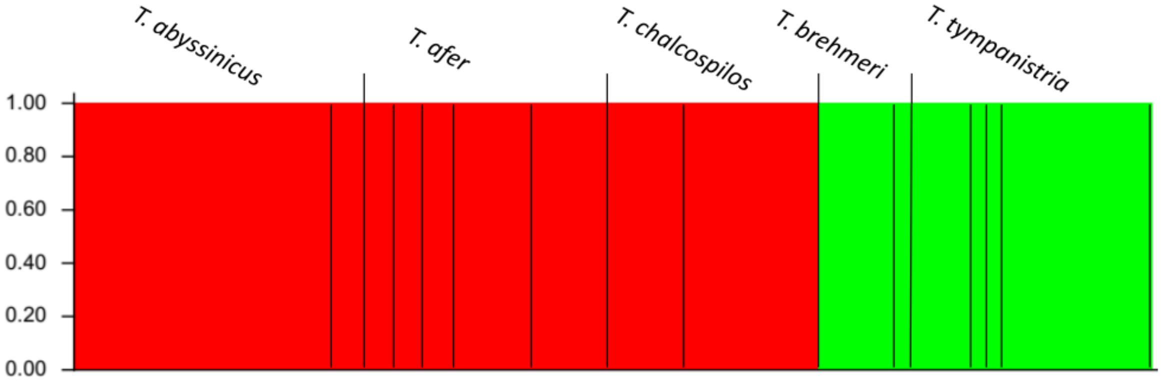


Figure 10. STRUCTURE analysis based on SNP data. Result for best K=2. Populations are separated by vertical bars and grouped by species.

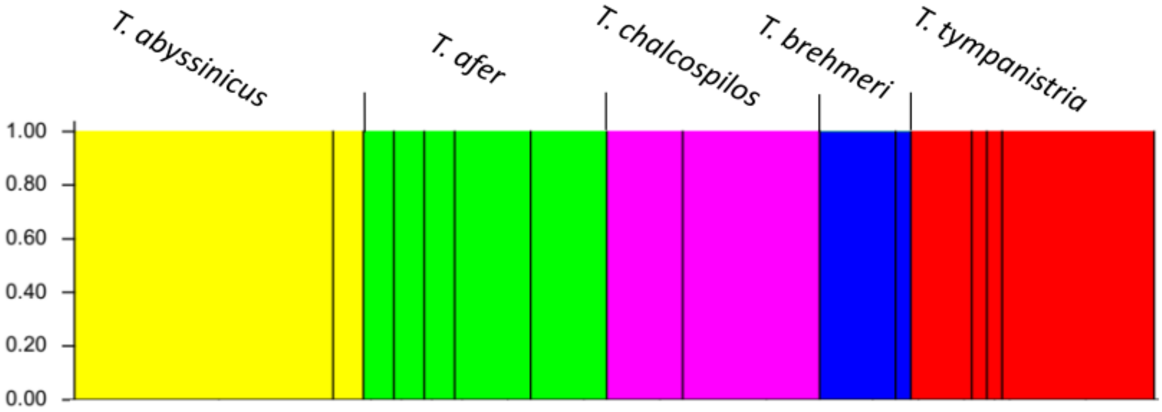


Figure 11. STRUCTURE analysis based on SNP data. Result for K=5 equal to the number of sampled species. Populations are separated by vertical bars and grouped by species.

4.3. Body size

We altogether caught 155 individuals of five *Turtur* dove species (from 11 to 50 individuals depending on species, see Supplement 2 for details). Studied *Turtur* doves significantly differed in many raw measurements of body size (see Supplement 2 for measurements and Supplement 3 for tests). To simplify the comparison of general body size variation among the studied species, we conducted principal component analysis (later PCA) based on all 7 original measurement variables (Table 1). The dataset was adequate for PCA, and we obtained a single component (PC) explaining ~73% of the original variables' variation (Table 1). One-way ANOVA with PC as the explained variable and species as a factor revealed high statistical differences between species ($F_{4,152} = 227.19$, $P < 0.0001$). Post-hoc Bonferroni comparison of all species pairs revealed significant ($P < 0.001$) differences, with a single exception. There was no significant difference between the general body size revealed by PC between *T. chalcospilos* and *T. abyssinicus* ($P = 1.0$).

Table 1. Principal component (PC) loadings for body size measurements of *Turtur* doves (N = 155 individuals). Kaiser-Meier-Olkin = 0.877. Bartlett's test of sphericity $\chi^2 = 1029.965$. $P < 0.001$.

Statistics and original response variables	PC
Eigenvalue	5.12
% of variance	73.12
Cumulative %	73.12
Wing	0.90
Tail	0.87
Tarsus	0.80
Beak length	0.73
Beak height	0.84
Head with beak length	0.91
Body mass	0.92

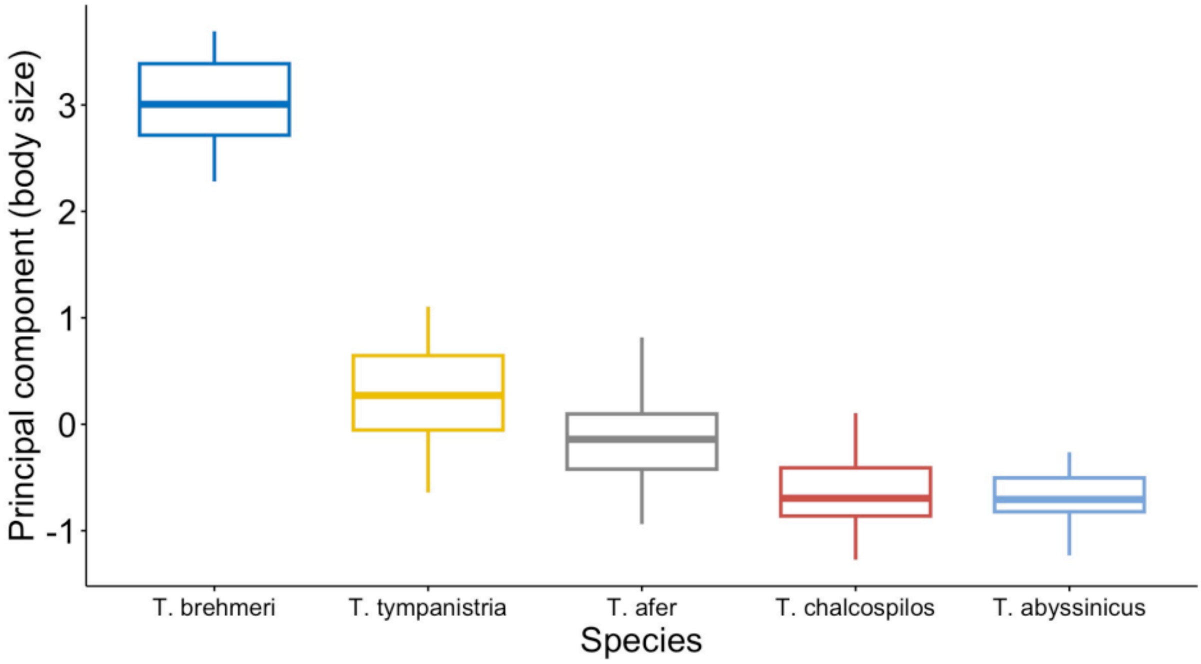


Figure 12. General differences in body size between *Turtur* doves are indicated by principal components derived from 7 body measurements (see Table 1).

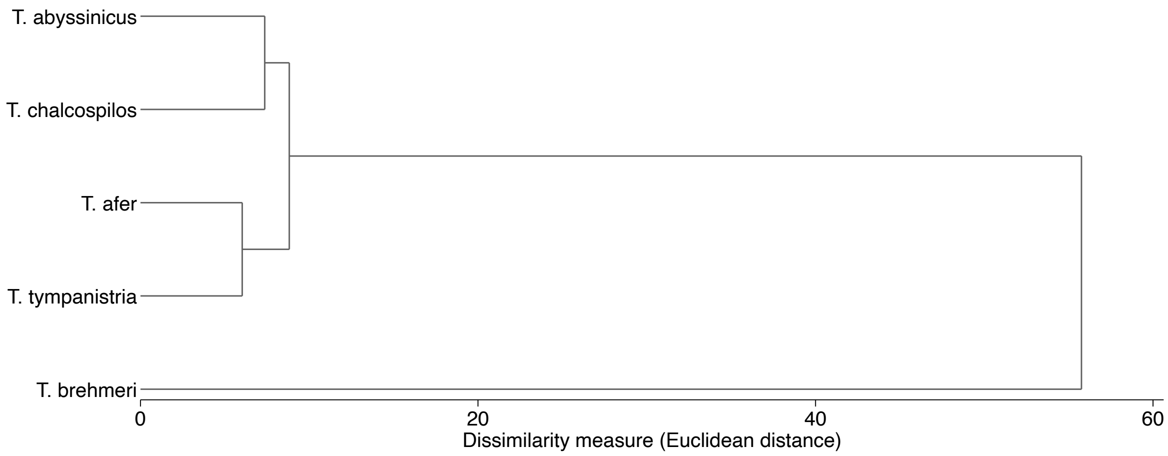


Figure 13. Dendrogram based on cluster analysis of all body measurements of *Turtur* sp. wood doves (complete linkage, Euclidean distance).

Cluster analysis of all body measurements confirms the clear separation of the largest *T.brehmeri* from all the other species (Figure 13). In general, the analysis of dove's body structures which may be correlated with sound production mechanism, namely the beak and the head, revealed no significant differences between three savannah species except the beak height. We found that the beak height of *T.afer* was slightly larger ($\bar{x}$ ± SD, 4.1 ± 0.24 mm) than that of *T. chalcospilos* (3.9 ± 0.24 mm), and significantly larger compared to *T. abyssinicus* (3.7 ± 0.27 mm) (Supplement 2 and 3).

4.4. Habitat preferences of the wood-doves

The comparative analyses of NDVI and EVI at sites inhabited by different wood dove species revealed significant differences between species. The entire models are presented in Supplement 4, while Figure 14 illustrates graphically predictive margins from both models, demonstrating differences between species. The subsequent cluster analysis (Figure 15) based on NDVI and EVI confirms our, based on literature, separation species into forest (*T. brehmeri* and *T. tympanistria*) and savannah species (*T. chalcospilos T. abyssinicus* and *T. afer*). Higher values of NDVI and EVI obtained for the first group well reflect forest preferences, but on the other hand, revealed that their habitat requirements are likely to overlap over a wide range of forest habitats.

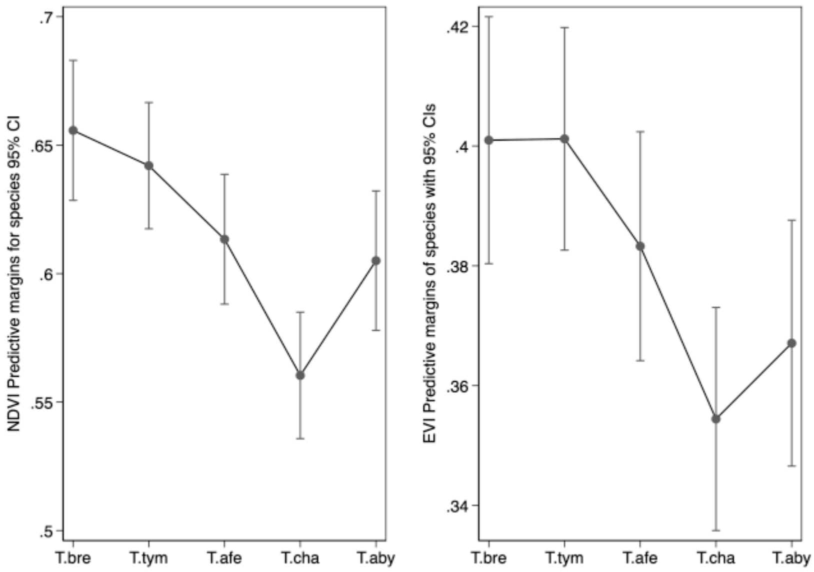


Figure 14. Predictive margins (95% Cis) of NDVI and EVI for studied wood dove species as a result of Gausian mixed models (see Supplement 4). Abbreviations: T.bre – *T. brehmeri*, T.tym – *T. tympanistria*, T.afe – *T. afer*, T.cha – *T. chalcospilos*, T.aby – *T. abyssinicus*.

The remaining three savannah species are separated into distinct cluster, however, *T. chalcospilos* branch separated quite early from the common branch of the *T. abyssinicus* and *T. afer* (Figure 15). These results confirm the separation of the studied wood doves into those that strictly prefer forest habitats and those that occur in a wide range of woodland and savanna-like habitats. Simultaneously, those 'savannah-like' habitats contain a wide range of habitats, from quite dense and structured miombo to open grassland.

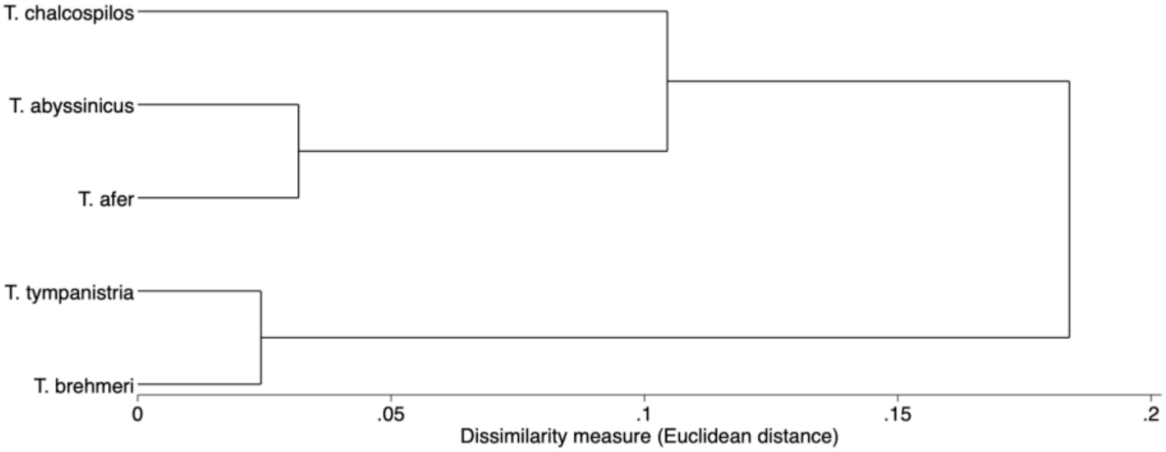


Figure 15. Dendrogram presenting cluster analysis based on NDVI and EVI characteristics of wood-dove species.

4.5. Song structure and between-species differences

Songs of all *Turtur* dove species low-frequency vocalizations, varying from 246 to 703 Hz (range between minimum and maximum frequency). Average song frequency is significantly lower (GLMM $\beta \pm$ SE 70.5±1.71, $z = 41.21$, $P < 0.001$) in larger forest species ($\bar{x} \pm$ SD, 447±25.3 Hz and 444±28.5, for *T. brehmeri* and *T. tympanistria*, respectively) than in smaller savannah species (538±26.8 Hz, 594±19.1 Hz, 583±25.7 Hz, for *T. afer*, *T. chalcospilos* and *T. abyssinicus*, respectively; Figure 17). Although the number of species compared is meagre, correlations between body size parameters and song frequency are negative (all below $r = -0.62$), and some are even significant (e.g. between tarsus length and average frequency $r = -0.92$, $P = 0.0246$). the bandwidth of songs is always very narrow (between 135 and 231 Hz in all species), and the differences in song frequency between individuals within a species differed slightly (up to 150 Hz). Songs produced by the same individual are generally reproduced with the same frequency parameters (see more details in Supplement 7).

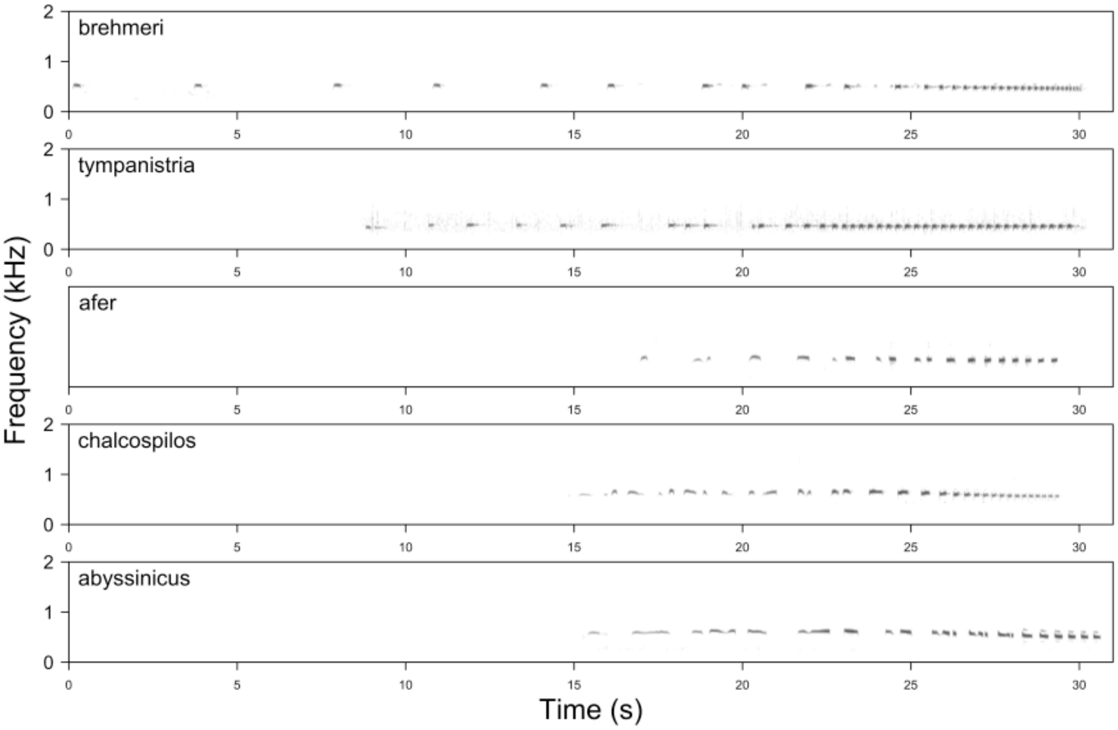


Figure 16. Spectrograms of songs of five studied *Turtur* dove species. To enhance the comparison of initial and final song phrases, the songs were aligned by the end. More examples enabling for within-species comparisons are presented in Supplement 7.

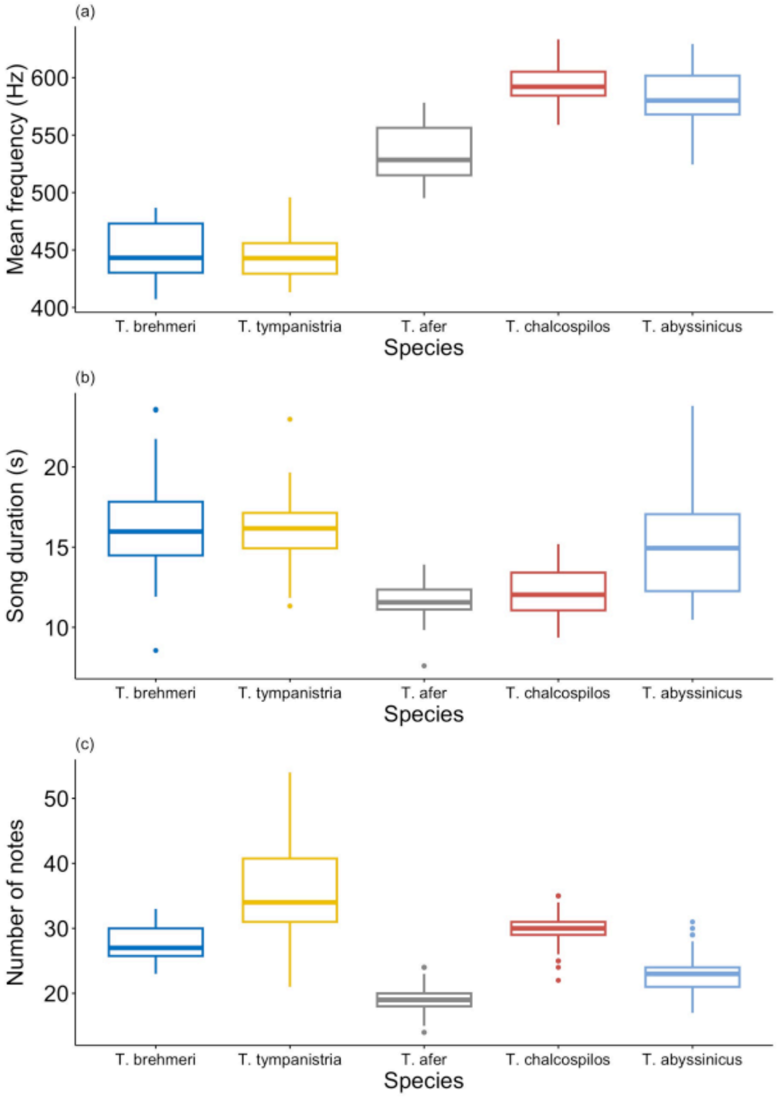


Figure 17. Box plots showing the average frequency (a), song duration (b) and number of notes within phrases (c) for all *Turtur* doves.

Song duration results from a common effect of notes and pause durations (Figure 17). In our sample, song durations varied from 7.6 to 23.8 s, but we know that sometimes songs may exceed even 30 s in *T. brehmeri* and *T. tympanistria*. Our dataset well reflects average values (ranging from 11.6 to 16.1 s) and within species variation, which is high. In all species, except *T. chalcospilos*, the longest song phrases are twice time longer than the shortest ones. In *T. chalcospilos* with minimum and maximum song durations ranging from 9.3 to 15.2 s, this variation is only a little bit lower. Number of notes in song phrases varied from 14 to 54, depending on the species. The most variable species was *T. tympanistria*, with songs ranging from 21 to 54 notes. In both forest species, the number and time pattern of notes produced in the initial song part seem more individually stable than in the final song part, with the opposite pattern in the three savannah species. Both in *T. brehmeri* and *T. tympanistria*, the number of syllables in the initial part is significantly (Table S 11) lower than in the final part of the song (Figure

18). On the other hand, in *T. afer*, *T. chalcospilos* and *T. abyssinicus* the number of notes in the initial part of the song is larger than in the final part (Figure 18).

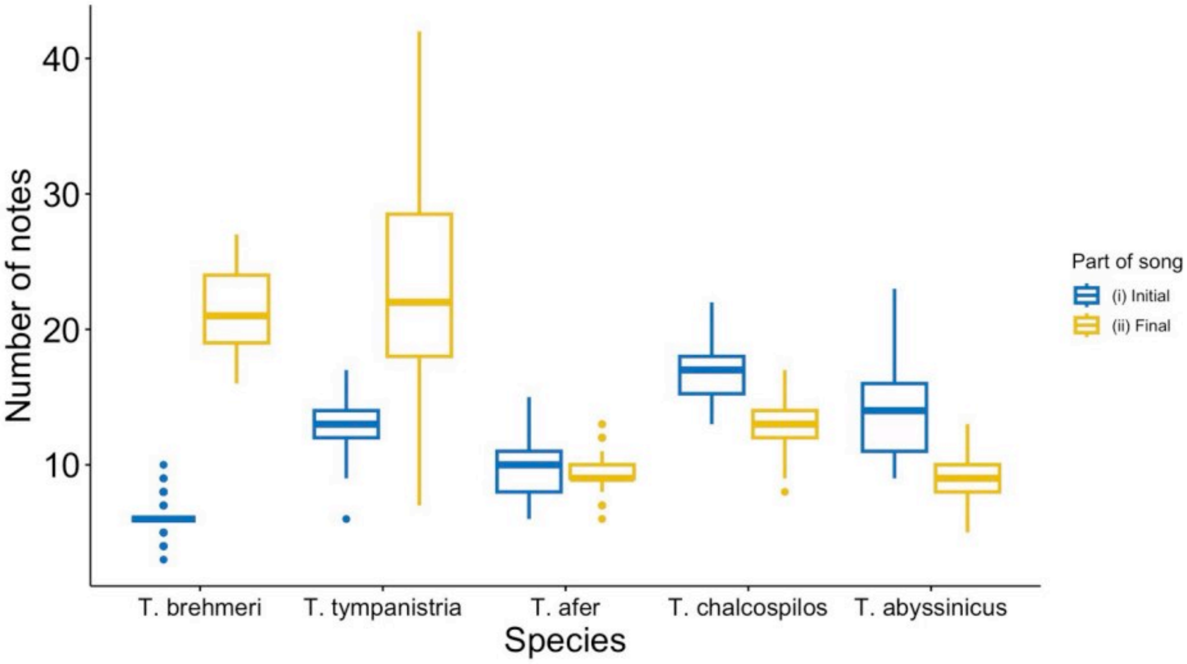


Figure 18. The number of notes within the initial and final parts of the song phrases in *Turtur* doves.

Cluster analysis based on entire song phrase characteristics indicates the separation of the forest and savannah wood doves into two groups. However, *T. afer*, seems to be more distinct than the other two savannah species.

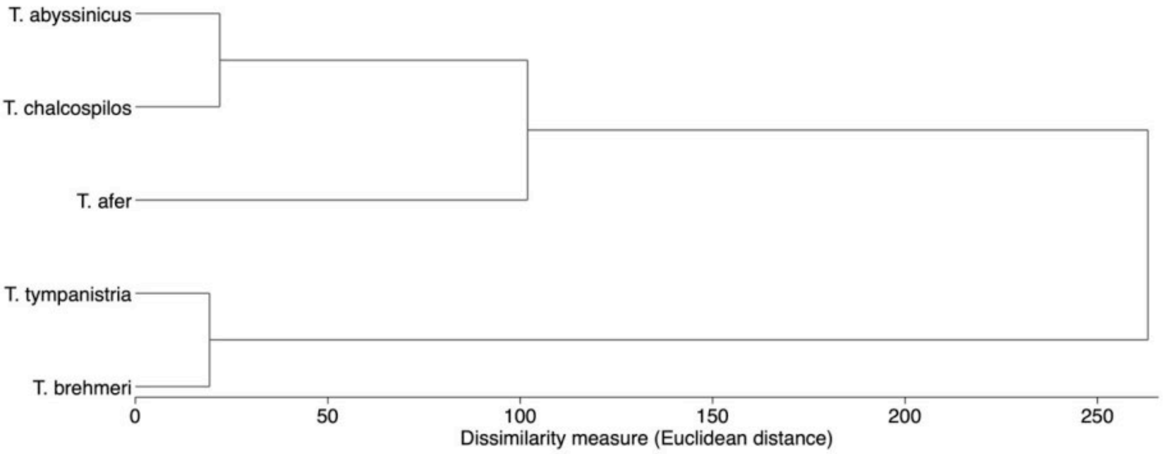


Figure 19. Dendrogram based on cluster analysis of entire song phrase measurements of *Turtur* sp. wood doves (complete linkage, Euclidean distance).

What is characteristic of all wood doves is that notes are always longer in the initial part than in the final part of the song (Table S 12, Figure 20). Moreover, durations of notes seem to be increasingly longer when comparing species across a gradient from acoustically closed to open habitats (Figure 20). This is one of the few characteristics found to be not significantly differentiated among species (Supplement 5). If, in the next step, we compare species differences in the time of pauses between notes, their values increase concerning the a priori assigned habitat attachment of each species (Figure 21).

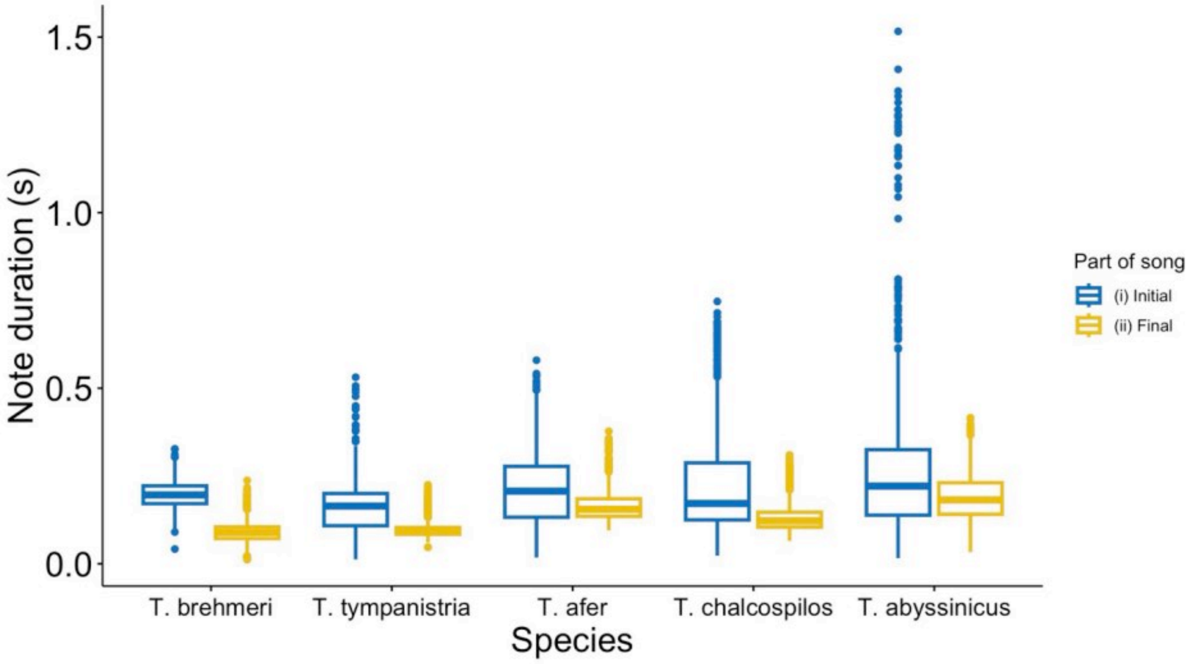


Figure 20. Note duration within the initial and final parts of the song phrases in *Turtur* doves.

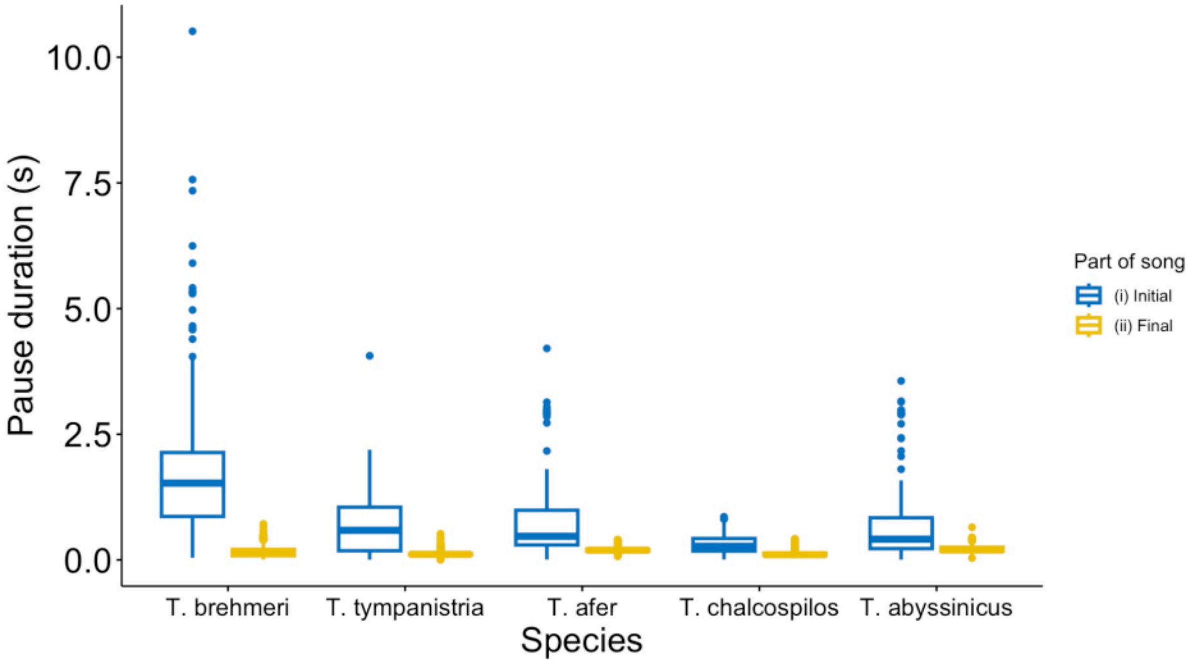


Figure 21. Pause duration within the initial and final parts of the song phrases in *Turtur* doves.

Time structure of song phrase measured with selection made by human observer in Raven Pro are potentially prone to human errors and depends on FFT window setting. To confirm that the time pattern described in such way is not biased we also calculated Pulse-to-pulse intervals which reflected time between automatically located peak of energy within notes. The pattern obtained matches the pause duration distribution very well (compare Figure 21 and Figure 22).

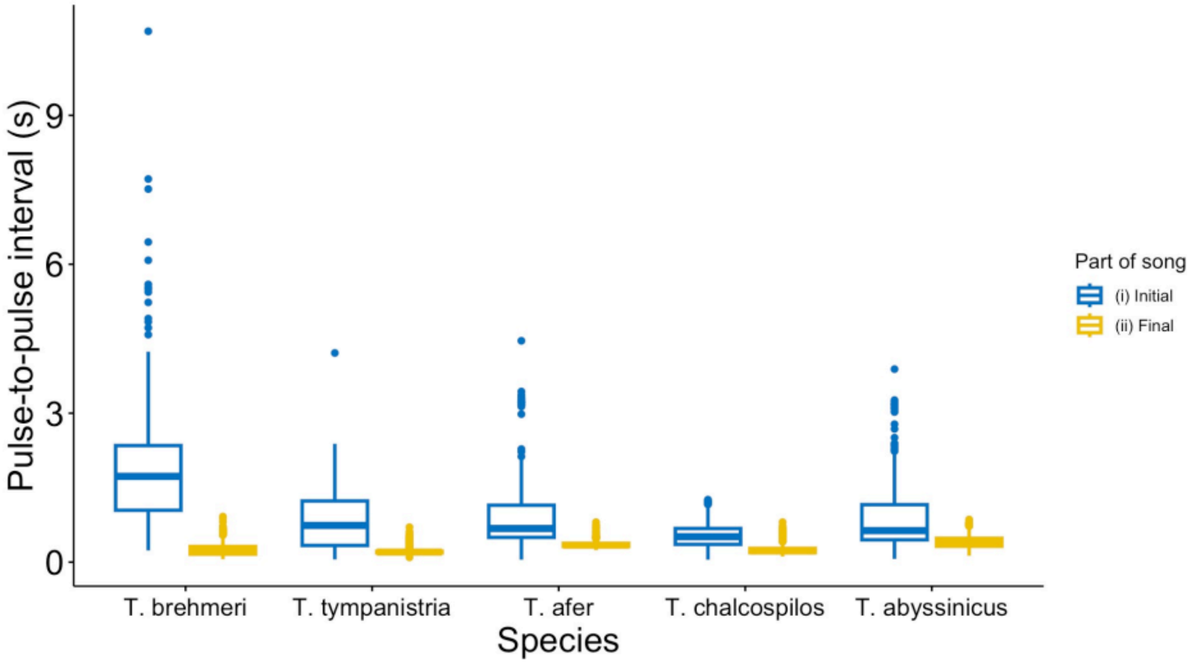


Figure 22. Pulse-to-pulse intervals within the initial and final parts of the song phrases in *Turtur* doves.

We found that in songs of all studied species the rhythm was more variable within the initial part than within the final song parts. The more detailed analysis reflects what we observed on sonograms (Figure 16) and indicates between species differences in the initial parts of the phrase, with rhythm decreasing pattern for *T. brehmeri,* irregular rhythm for *T. tympanistria*, and differentiated but regular patterning in all savannah species. These differences are well visible when several examples of songs from different individuals of the same and different species are compared (see Supplement 7).

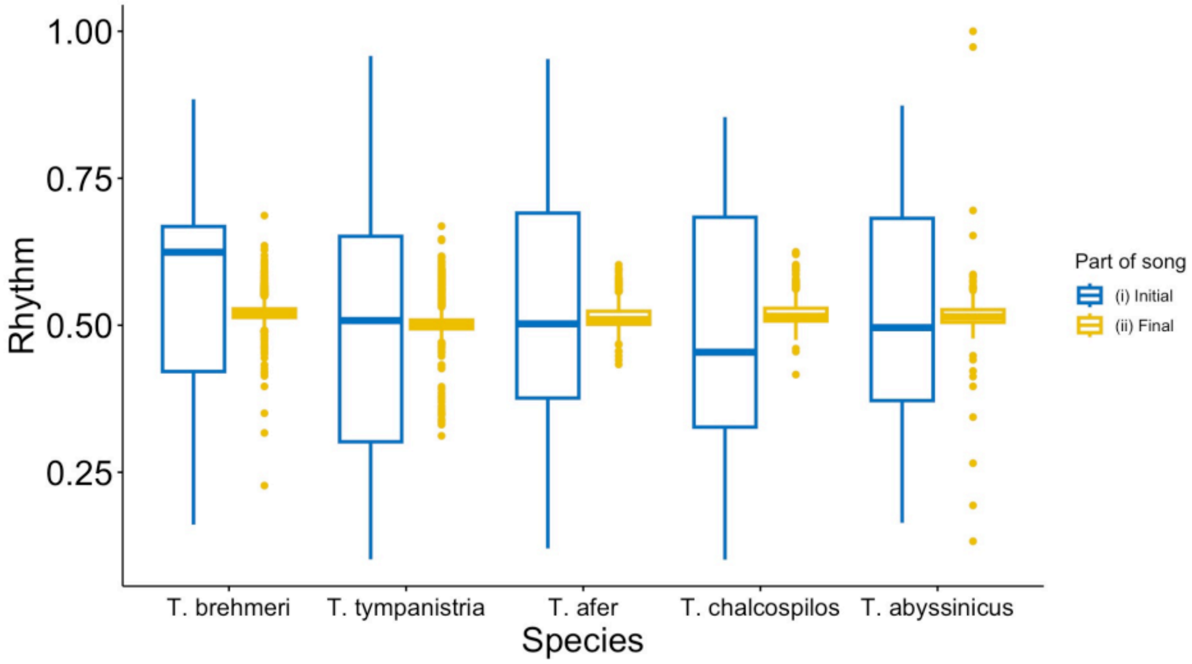


Figure 23. Variability in rhythm within the initial and final parts of the song phrases in *Turtur* doves.

Some parameters of the song, such as peak frequency of notes (Figure 24) or relative amplitude (Figure 25), show apparent differences between species, creating two groups of birds. Forest doves have, on average, a lower maximum frequency in both parts of the song and a higher relative amplitude in the initial part of the song than savannah doves have (see Figure 24, Figure 25).

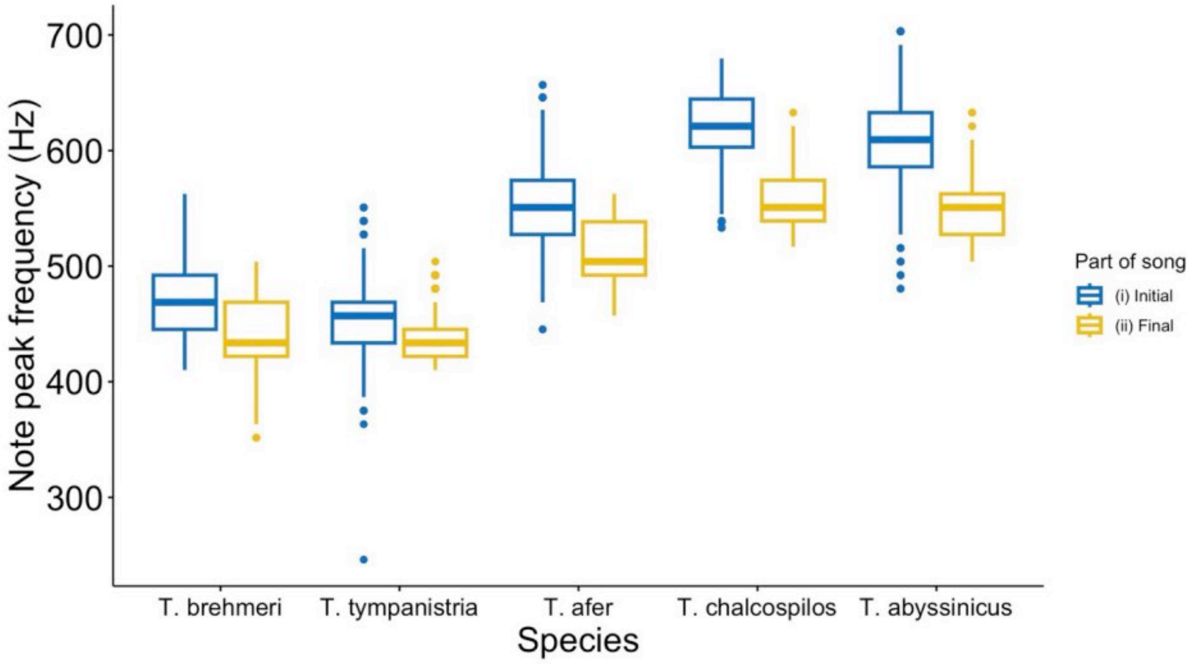


Figure 24. Variability in note peak frequency within the initial and final parts of the song phrases in *Turtur* doves.

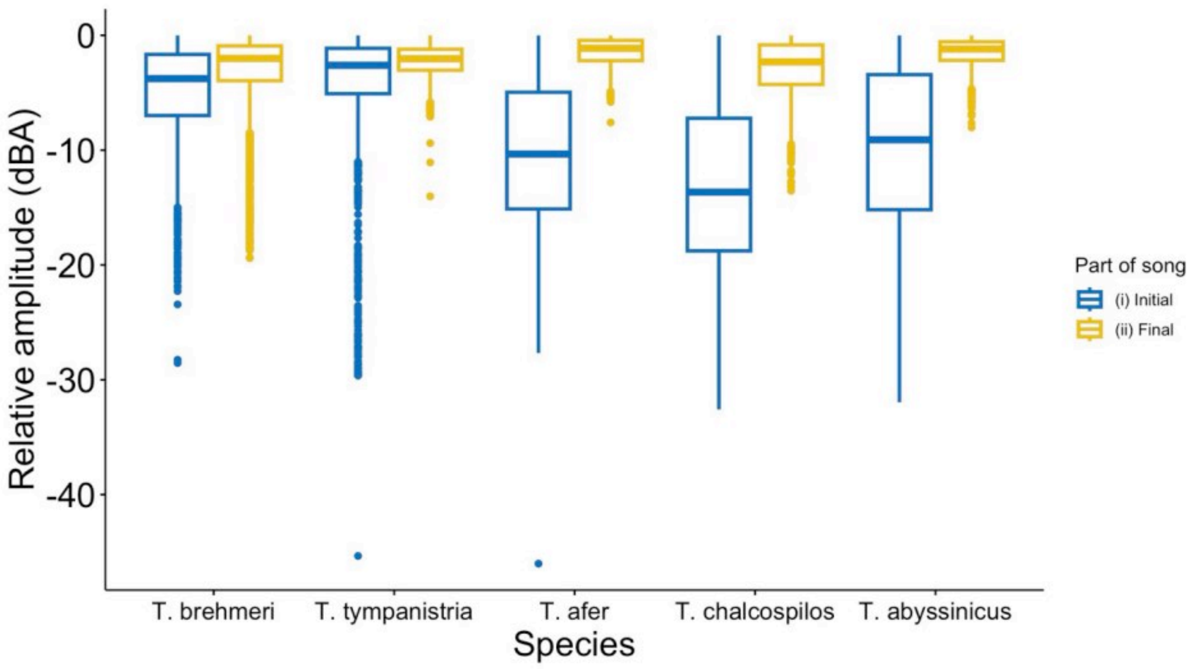


Figure 25. Variability in note relative amplitude within the initial and final parts of the song phrases in *Turtur* doves.

The song's structure is common across the genus, meaning that in all five species, we can recognise an initial and final part of the song. The initial part has longer notes (Figure 20), as well as longer pauses between them (Figure 21), which are greatly shortened and unified in the final phase of the song. The length of the notes and the pauses between them is also more varied in the first part. Pulse-to-pulse intervals (Figure 22) show the same trends, i.e. they are longer and more varied at the beginning of the song, while in the final part, they look very similar for all species. The note peak frequency (Figure 24) is higher in the initial part than in the final part of the song. The relative amplitude (Figure 25) has the opposite pattern in all species and is lower in the initial part of the song than in the final part. However, the difference between the relative amplitude of song parts is much smaller in the first two *Turtur* species inhabiting the forest. The last parameter measured was rhythm (Figure 23), which is very variable in the first part of the song giving the illusion of its absence, while in the final part it is distinct and at a similar level in all species. A detailed inspection of the rhythm indicates that it is clearly changing throughout the initial part of the song and remains stable and similar in the final part. The characteristics of rhythm changes in the initial parts of the song are diversified among species and seem to be one of the species-specific characteristics for each of them.

Figures (Figure 26, Figure 27) combine information about song parameter changes with the following notes or time and allow for a better understanding of how the songs of different *Turtur* species differ from each other and how they change while they are produced. We can see from these figures that some song parameters have roughly similar courses over time, while some have different patterns.

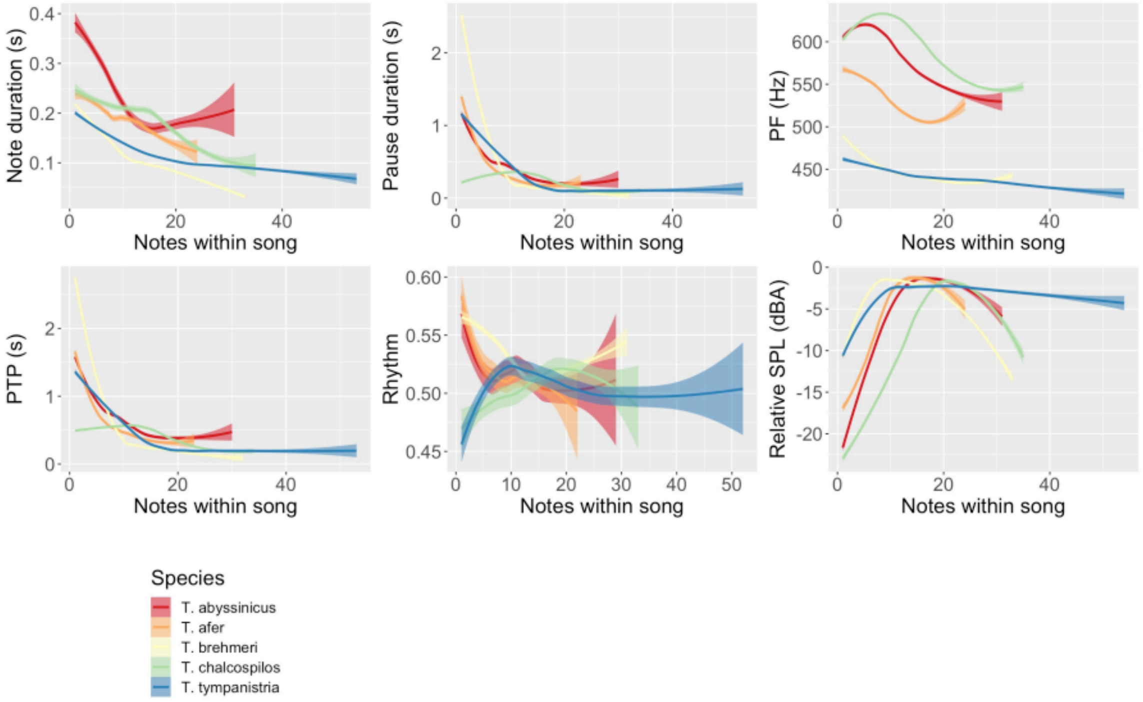


Figure 26. Species averaged changes (with "loess" function - locally weighted scatterplot smoothing) of song parameters with the course of phrase indicated by the sequence of notes.

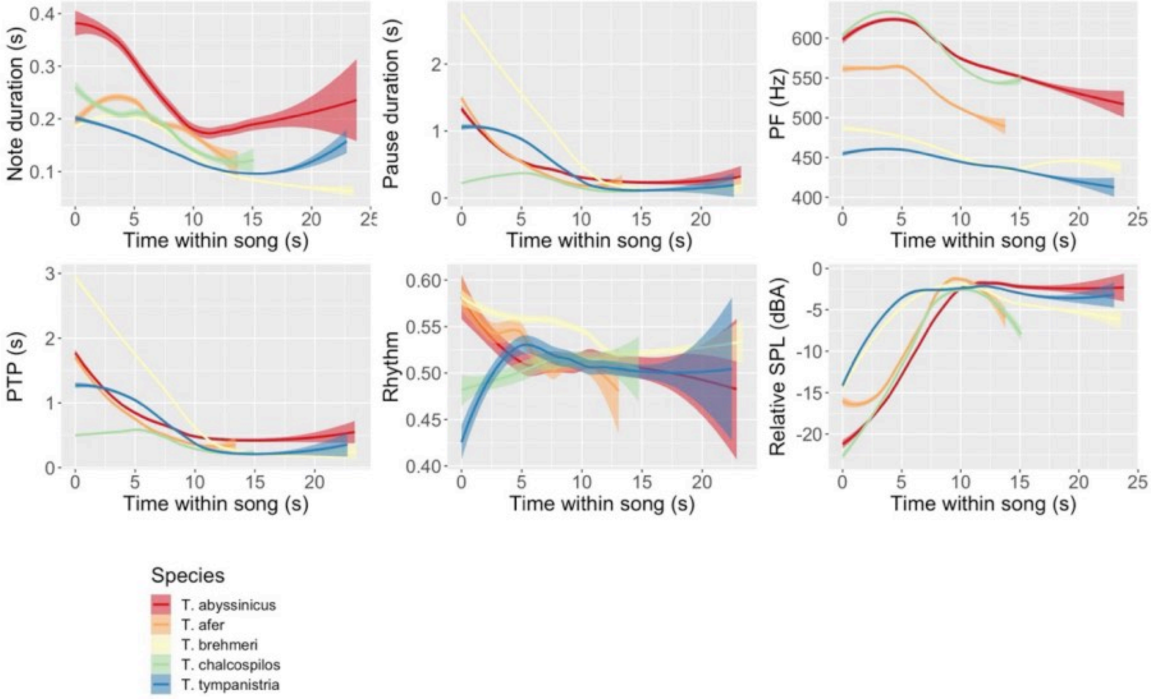


Figure 27. Species averaged changes (with "loess" function - locally weighted scatterplot smoothing) of song parameters with the course of phrase indicated by the time within phrase.

In the case of note duration, forest species have them considerably shorter than savannah species. Still, a general tendency to shorten notes as the song progresses is noticeable in all species. The duration of the pauses between notes, as well as the pulse-to-pulse duration at the beginning of the song phrase, are longer in forest doves than in savannah doves, but by the end of the song phrases, they shorten to a similar level.

Between-species differences in note and pause durations (and hence also in pulse-to-pulse intervals) in the initial part of songs result from syntactical differences, which seem to follow habitat preferences from forest to the most open savannah. In *T. brehmeri,* we have simply shortened pauses (and, to a lesser degree, notes) with time. In *T. tympanistria*, shortening is also a general rule, but notes tend to group in two or three together, forming an individually specific (see further) pattern. In savannah species, notes in an initial part form more or less regular pairs of a shorter and longer note, and longer notes are longer, the more open the habitat a species prefers for a living. The biggest and the easiest to notice is the difference in the peak frequency of the following notes, where savannah doves reach a much higher pitch than wood doves. The song's rhythm is variable at its beginning and varies by species; common to the genus is only its stabilisation towards the end.

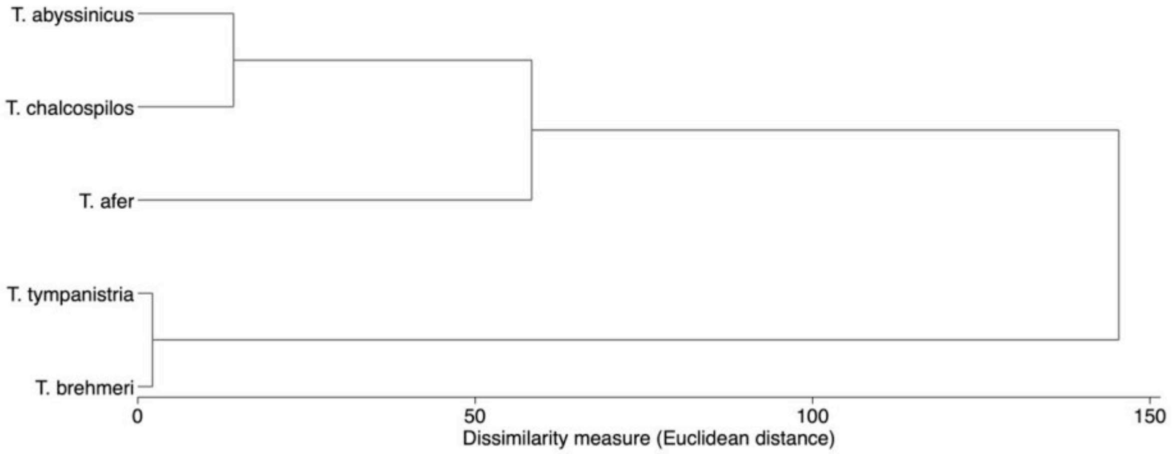


Figure 28. Dendrogram based on cluster analysis of notes and pauses of initial part of the song phrase measurements of *Turtur* sp. doves (complete linkage, Euclidean distance).

Cluster analyses with parameters of initial song parts (Figure 28) or final song parts (see Figure 29), produced very similar dendrograms, confirming again separation of forest and savannah species.

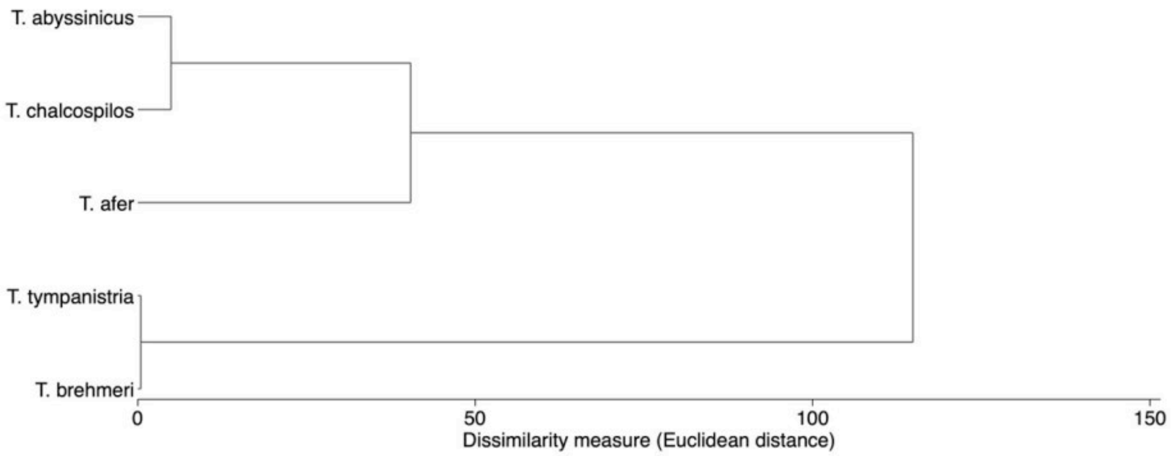


Figure 29. Dendrogram based on cluster analysis of notes and pauses of final part of the song phrase measurements of *Turtur* doves (complete linkage, Euclidean distance).

The relative amplitude of notes within song phrase is low at the beginning in all species. It increases in a parabolic pattern. However, it seems to be more stable and higher in some species (i.e. in both forest doves and in *T. abyssinicus*) if we consider course over time rather than a course over following notes. There is also a clear division between forest and savannah doves, where the sound amplitude is higher in forest doves. These results are presented in the next chapter.

4.6. Amplitude of song and song active ranges

We measured song intensity and song detection distance of all studied species, although the sample sizes are small. In *T. brehmeri*, songs had a mean amplitude of 82 dB at a distance of 1 m (79–85 dB, N = 16). In *T. tympanistria,* the average amplitude was 80 dB at a distance of 1 m (78–85 dB, N = 6).

In the case of *T. brehmeri* detection of male song was possible from a distance of 400-500 m, depending strongly on terrain configuration (N = 2). The same values were obtained for *T. tympanistria* (N = 3), however once we had a better opportunity to measure a male position in a flatter area, male was heard from at least 270 m. Despite relatively low typical song amplitudes, both forest wood doves could be heard from 270 to even 400-500 m (Figure 30, Figure 31).

In the case of all savannah species, all amplitude measurements were much lower. In *T. chalcospilos* (N = 18) and *T. afer* (N = 4), both ranges and means were very similar, 69 dB (58–72 dB) and 70 dB (67-73 dB), respectively. The amplitude of *T. abyssinicus* seems to be even lower, and while measured, it oscillates around 55–68 dB with an average of around 65 dB (N = 2). We could detect all Savannah species from 150-250 m (Figure 30, Figure 31). In summary, for rudimentary calculations, we may assume that forest species typically sing with amplitude around 80-82 dB, while in the savannah, it is instead 65-69 dB. Such intensities enable detecting songs (for humans) from up to 500 m in the forest and 250 in the savannah in optimal conditions.

To predict how the relative amplitude affects the active range of the species' songs, we assume that the longest distance the song of a species could be heard results from the intensity of the loudest notes within a song phrase (i.e. zero values of Relative Amplitude of a particular phrase). Then we recalculated the probable hearing range of each note using the inverse square law of sound propagation, which states that each doubling of distance from a source decreases sound intensity by 6 dB. Such calculations are very simple and do not consider many factors, including – for example – energy loss resulting from habitat density, obstacles etc. However, we used them only to compare the songs of different species and how distinctive parts of songs differ in their maximal propagation potential. We present the results of this modelling in Figure 30 and Figure 31.

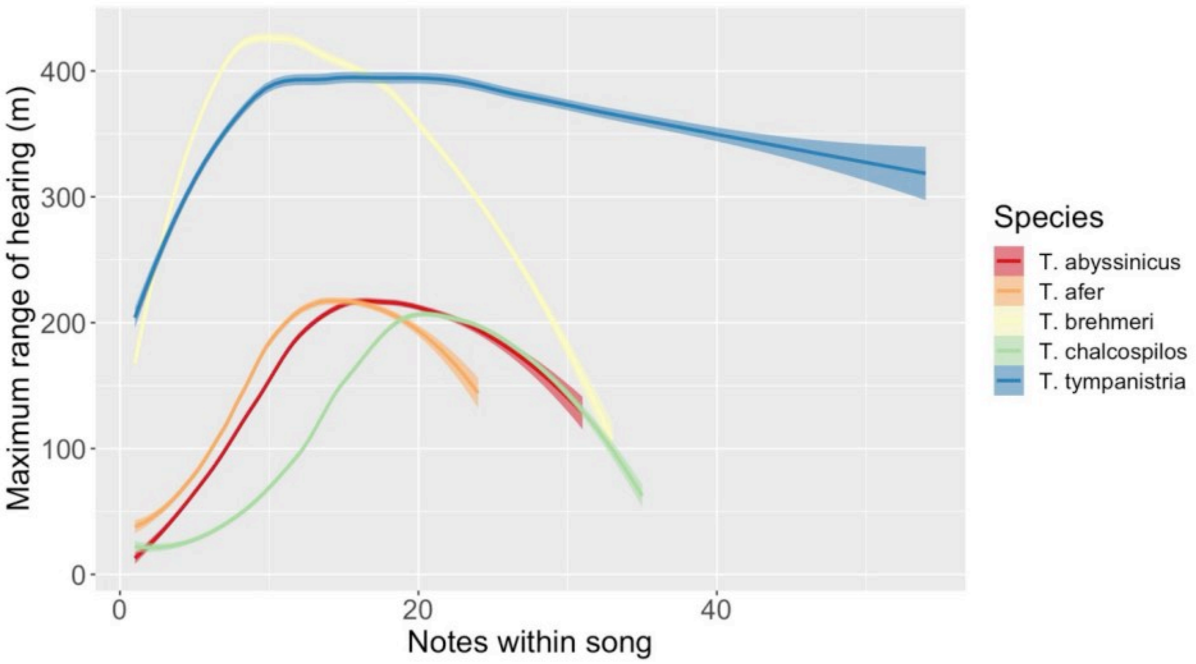


Figure 30. Averaged ranges at which specific notes within the *Turtur* dove's song phrase can be heard (“loess” function - locally weighted scatterplot smoothing).

There is no doubt that forest and savannah species have entirely different maximum hearing ranges, which were also measured directly in the field (400-500 m for forest species and 200-250 m for savannah species). However, what seems more important is that the active range of different parts of the song in all species is quite variable, and sometimes the relatively short part of the song phrase reaches the maximum. In the case of both forest wood doves, the maximum hearing range is reached between 7 and 12 s of the phrase and remains only slightly lower till the end of the song. In all savannah wood-doves, the beginning of song phrases have very low amplitudes, which increase slowly, reaching maximum after 9-11 s, and in *T. afer* and *T. chalcospilos*, only for a very short time. With this respect, *T. abyssinicus* differ from savannah congeners as the maximum amplitude of the song is kept till the end of the song phrase (see Figure 31).

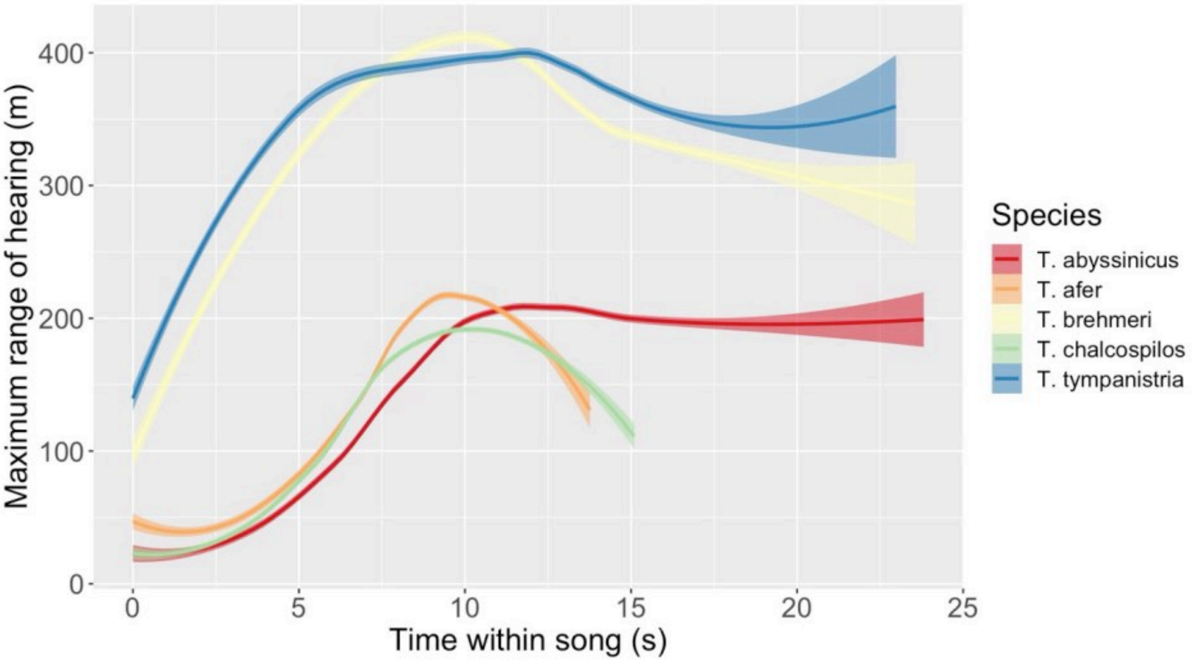


Figure 31. Averaged ranges at at which specific parts (indicated by the time within phrase) within the *Turtur* dove's song phrase can be heard "loess" function - locally weighted scatterplot smoothing).

4.7. Potential for species-identity coding

In the first set of analyses, we aimed to determine which of the entire song features allow for discriminating between different species of *Turtur* doves. We found that the number of notes comprising a song and song phrase duration did not allow to discriminate between species reliably. In fact, only 58% and 39% of song phrases (respectively) were appropriately assigned to the species; details in Table 2) were also indicated by very low values of H_s. However, repeated analysis with the number of notes counted separately for the initial and final parts of the song phrase (H_s = 1.71) significantly improved the discrimination, reaching 76% of correct assignments. The frequency parameters of the song also have quite a considerable potential for species discrimination (69%) with H_s = 2.04. The final analysis, including all seven song phrase variables, allowed 90% correctness of discriminating between species with H_s value 2.15.

To sum up, it seems that song duration and related to it number of notes within a song have a small potential for being used for own species recognition. However, the proportion of notes between the initial and final song parts and song frequency are much more useful for assigning a phrase to a particular species.

Table 2. Potential for species-identity coding in the entire song phrase characteristics of *Turtur* dove species.

Song phrase characteristics	Hs	DS
Number of notes within song	0.31	0.58
Number of notes in Initial and Final part	1.71	0.76
Song duration	0.29	0.39
Song frequency (min, avg, max)	2.04	0.69
All parameters	2.15	0.92

In the next step, we compared species discrimination based on 1–8 note sequences related to the beginning and end of the song phrase. The pattern revealed in this comparison indicates relatively high and similar values of H_s (between 1.87 and 2.35). However, they significantly differed between song parts and were much less variable and higher in the final song part (GLMM $\beta \pm$ SE 0.075±0.338, z = 2.22, P = 0.027). It seems that regardless of the relatively uniform spread of species-specific information within song phrases, the most consistent (invariant within a species) information was encoded in the final part (Figure 32).

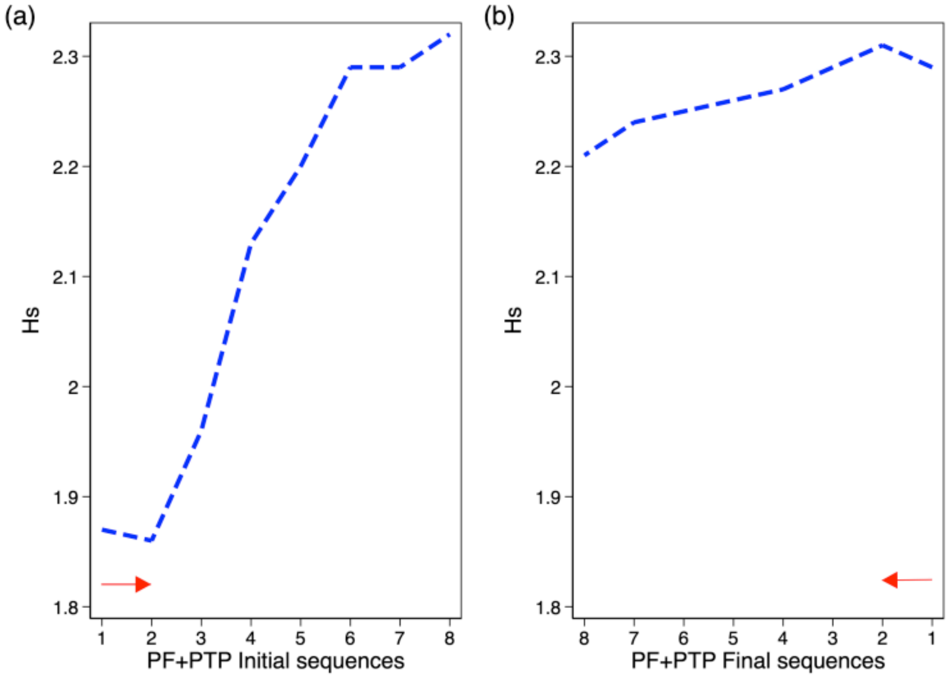


Figure 32. Values of Beecher's information statistics H_s calculated for 1 to 8 note sequences within song phrases, starting from the beginning or end of phrases (indicated by red arrows). Each sequence consists of PTP+PF values and identity variables containing information about belonging to a species (1 to 5). Based on 500 phrases, 10 phrases per male and 10 males per species.

These results suggest relatively short sequences of notes (especially at the end of the song), with their specific distribution in time and characteristic frequency, should allow for efficient discrimination between species.

4.8. Potential for individual-identity coding

4.8.1. Song-level parameters

Applying the same procedures for each species separately and testing for efficiency in individual discrimination within a species revealed a diversified pattern (Supplement 6). The discriminant analysis revealed little or no potential for identity coding when we only considered the number of notes within a song or song duration. The frequency of song phrases was much more individually specific and including all variables improved discrimination (i.e. higher H_s and *DS* values than for single measures, Supplement 6). Values of H_s suggest that songs are very individual in *T. tympanistria* ($H_s = 3.47$) and *T. chalcospilos* ($H_s = 3.07$) even if we consider only the entire song phrase characteristics. Slightly worse was *T. abyssinicus* ($H_s = 2.8$), while both *T. brehmeri* ($H_s =1.71$), and *T. afer* ($H_s = 1.12$) seem to be individually not specific at the level of whole phrase variation. To sum up, even if the whole song phrase characteristic is considered, it seems that in some species it has a high potential of discriminating between different individuals, with song frequency contributing the most to the discrimination.

4.8.2. Note-level parameters

In the analysis of the potential for identity coding within song phrases, we focused on 1 to 8 note sequences and PTP+PF distribution. The patterns of Beecher's information statistics that we found for different species, song parts, and sequences are illustrated in Figure 33. Species significantly differed in their potential for identity coding as measured with Beecher's information statistics (Kruskal-Wallis rank test $\chi^2 = 62.257$, d.f. = 4, $P = 0.0001$). The most individually specific songs were found in *T. tympanistria*, followed by *T. abyssinicus* and *T. brehmeri*, while both *T. afer* and *T. chalcospilos* had the least individually specific sequences. We found that initial parts of songs were more individually distinctive (H_s 1.91±0.83) compared to the final part (H_s 1.59±0.67). In some species, individuality increased with the duration of the sequence, most notably in the initial parts of the songs of *T. tympanistria* and *T. abyssinicus*. However, H_s values were less variable for the final part of the song and in *T. afer* and *T. chalcospilos* did not increase with sequence duration at all (GLMM $\beta \pm$ SE -0.34 ± 0.097, $z = -3.34$ $P = 0.001$).

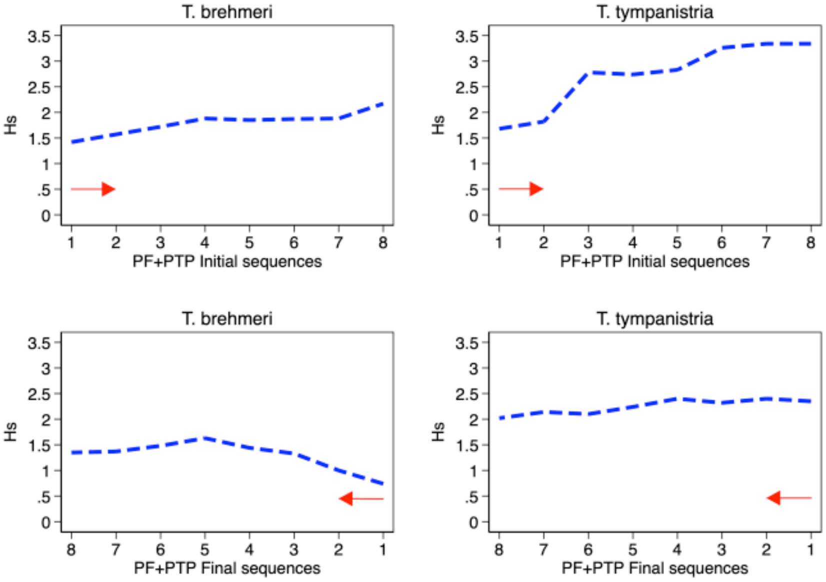


Figure 33. Values of Beecher's information statistics Hs calculated for 1 to 8 note sequences within song phrases of two forest wood-dove species. Each sequence consists of PTP+PF values and identity variables containing information about belonging to a male (1 to 10). Based on 100 phrases in each species (10 males x 10 song phrases per male).

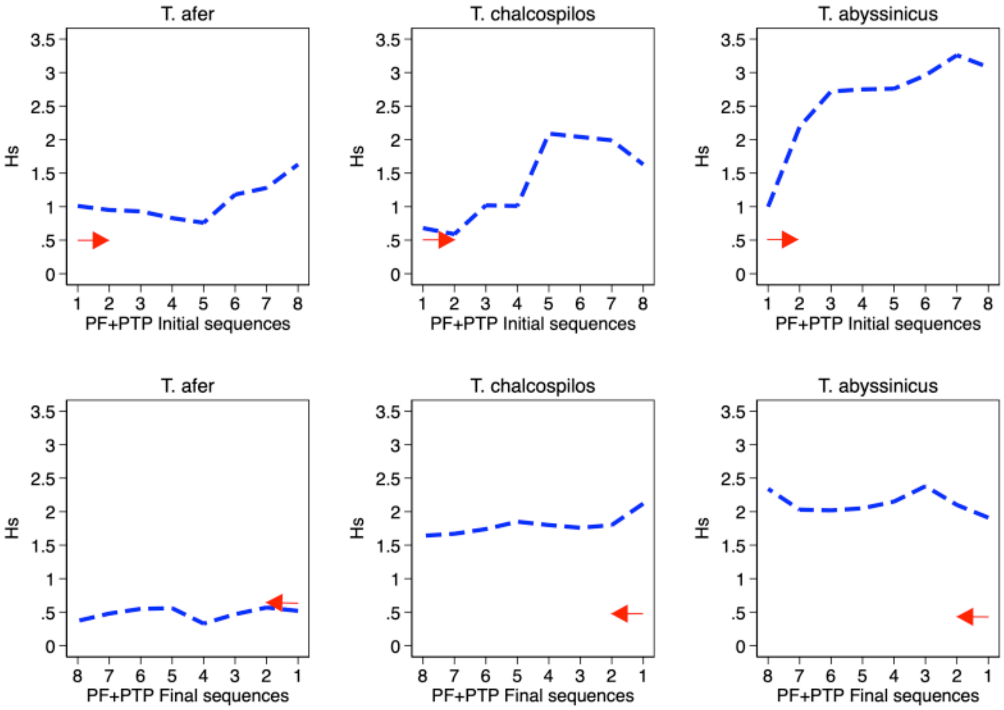


Figure 34. Values of Beecher's information statistics H_s were calculated for 1 to 8 note sequences within song phrases of three savannah wood-dove species. Each sequence consists of PTP+PF values and identity variables containing information about belonging to a male (1 to 10). Based on 100 phrases in each species (10 males x 10 song phrases per male).

5. Discussion

5.1. Summary of results

This study, for the first time, provides a detailed description of the songs of doves belonging to the *Turtur* genus. The songs of the studied species share a common genus syntax, and their songs consist of two parts with distinct patterns in the initial and final sections. The initial part of the song exhibits greater variability, higher frequency, and longer (and/or more variable in duration) notes and pauses between notes, as well as changes in rhythm. Variations in frequency and note patterns among wood dove songs are related to morphology, phylogeny, and habitats of occurrence. While there is strong support for hypotheses suggesting such relationships (MAH and AAH), exceptions indicate possible intense selection pressures leading to a reduction in song pitch in *T. tympanistria*. Additionally, songs of all species display significant individual specificity, offering an opportunity to study the factors shaping the evolution of acoustic identity in non-learned birds through song production.

5.2. Phylogeny, morphology, and habitat

The phylogenetic analysis revealed that genus *Turtur* is divided into two well-supported lineages, which consist of species preferring different types of habitats. The first group of forest wood doves consist of *T. brehmeri* and *T. tympanistria*, and the second – savannah group – consist of *T. afer*, *T. chalcospilos* and *T. abyssinicus*. The two forest wood doves were found to be genetically much different from each other than all savannah species. This is supported by biometry and other morphology details. Both forest species are larger than savannah species, while still *T. brehmeri* is much larger than *T. tympanistria*. On the other hand, the colouration of *T. brehmeri* and *T. tympanistria* is entirely different, and only *T. tympanistria* has apparent sexual dimorphism revealed in feather colouration (Baptista, Trail, Horblit, & Boesman, 2020b; Kirwan et al., 2021). All savannah species are very similar, with *T. afer* being slightly but significantly larger than *T. chalcospilos* and *T. abyssinicus*. One may say that they differ in detail by the meaning of feather, beak and leg colouration. Nevertheless, all species have iridescent feather patches on greater coverts and tertials, which substantially differ between species.

A comparison of body measurements indicated that *T. brehmeri* is much more distant from the other congeners, with body size significantly larger in any characteristic analysed. Cluster analysis based on biometry revealed that the remaining four species form two branches, with slightly larger *T. tympanistria* and *T. afer*, and the smallest pair of species: *T. chalcospilos* and *T. abyssinicus*.

The cluster analysis based on NDVI and EVI derived from sites inhabited by particular species confirms the distinction between the forest and savannah wood doves. However, the closest pairs of species overlap in habitat preferences, and our analysis fits the field observations of co-occurring species in several habitats. Simultaneously, this analysis suggests that by the meaning of vegetation variation, savannah species occupy a wider range of habitats. *T. brehmeri* occurs only in lowland primary forests or old secondary forests and is restricted to elevation below 750 m asl. It avoids any cultivated areas or disturbed forest patches, which indicates that everywhere in the species range, it sings in a similar type of closed environment (Baptista, Trail, Horblit, & Boesman, 2020b). As lowland African forests belong to one of the most stable and long-lasting habitats on Earth (Corlett & Primack, 2011), a reasonable assumption is that *T. brehmeri* produce signals that perfectly match this environment's parameters for optimal transmission of information.

The second forest species, *T. tympanistria*, has a much wider species range, and despite the fact that there is a significant overlap between its environmental requirements and the requirements of *T. brehmeri*, it seems that that *T. tympanistria* occurs in any kind of forest-like habitat from the sea level to 3000 m asl. It could be found in natural primary lowland and mountain forests, as well as in secondary forests, riparian thickets, gardens, plantations, forest clearings, and forest edges (Kirwan et al., 2021). We observed single males *T. tympanistria* also on savannah, for example, in Lake Mburo NP, where it occupies patches with solitary larger trees surrounded by dense bushes. Concerning acoustic signalling in such a range of habitats, the only thing that can be said is that, indeed, in the evolutionary time scale, this species came into contact with acoustically different environments. Although undoubtedly, it was dominated by those structurally complicated and acoustically closed.

All the ‘so called’ savannah species prefer more open habitats, and although different in habitat preferences, they may co-occur in several environments across Africa. *T. afer* occurs in moist woodland, secondary forest, and forest edges, including any anthropogenic habitats, like *Eucalyptus* plantations, and up to 2000 m asl. However, it avoids primary forest (Baptista, Trail, Horblit, & Boesman, 2020c). In practice, we recorded *T. afer* at the border between forest and plantation in Ghana, or in denser patches of savanna in Uganda. *T. chalcospilos* occurs in different types of woodland savannah, miombo, lowland riparian forests or cultivated areas (Baptista, Trail, Horblit, Boesman, et al., 2020a). Similar to T. afer, it prefers less dry areas than the last species in this group, *T. abyssinicus*. The most typically, *T. abyssinicus*, occurs in savanna woodland with *Acacia* and *Combretum* trees; however, it can be found wherever around the desert zone, where the existing vegetation (e.g. plantations) and access to water make minimal requirements to survive (Baptista, Trail, Horblit, & Boesman, 2020a). To sum up, all savannah species live in various environments that are generally less dense, less vertically stratified, but more variable horizontally than those in which forest doves occur. The other important aspect of acoustic communication is that these species cannot sing from a very elevated song post (e.g. in Kakum NP forest, trees had up to 50-60 m). Thus, savanna species usually sing from posts having maximally 5-10 m (often even less), and their songs have to propagate through patches of dense shrubs preceded by unobstructed terrain (grassland). Unequivocally, species from the two distinguished environmental groups face different challenges in transmitting their singing through the environment.

5.3. Song characteristics – which are shared, which are different?

The most apparent and commonly shared song characteristic of all studied wood doves is a song phrase consisting of two distinct parts, which we called initial and final. It has to be considered an ancient characteristic of the genus, especially as the closest relatives have different song structures. Two-part songs are, however, quite commonly found across different bird species, including some members of Columbidae. For example, in the sula cuckoo-dove (*Turacoena sulaensis*), the song phrase consists of a series of short notes (~20-30 ms), very similar to those in the initial part of forest wood doves, followed by much longer (~100-300 ms) whistles. All these notes are produced with low frequency around 1 kHz (Baptista, Trail, Horblit, & Kirwan, 2020c). However, while the second species from this genus, the slaty cuckoo-dove (*T. modesta*), exhibit also double-part phrase characteristics (but less prominent), the third southeast Asian member of the genus, white-faced cuckoo-dove (*T. manadensis*) has much more typical "pigeon" song consisting of repeated sound without any clear phrase syntax. The closest relatives of *Turtur* doves, like the Namaqua dove (*Oena capensis*) from Africa and emerald doves from Asia (genus *Chalcophaps* with three species), do not share double-structured song phrases and sing phrases consisting of repeated syllables (Baptista, Trail, Horblit, & Kirwan, 2020b; Baptista, Trail, Horblit, Kirwan, et al., 2020d, 2020e; Del Hoyo et al., 2020).

The second common feature of the studied wood dove songs is low frequency and very narrow bandwidth. Song frequency in between-species comparison usually matches predictions of MAH and AAH hypotheses (Farina, 2014). MAH predicts the negative correlation between body size and song frequency, where larger species are capable of producing lower-frequency sounds (Fletcher, 2004; Friis et al., 2021; Mikula et al., 2021; Ryan & work(s):, 1985; Wallschläger, 1980; Wiley, 1991). This relation is explained in birds by the fact that only a small apparatus (the syringeal membrane) can produce high-frequency sounds (Farina, 2014). AAH stresses the importance of environmental constraints and assumes that sounds used in long-range communication should match the habitat acoustics to maximise the transmission efficiency (Morton, 1975). Low-frequency songs travel through the vegetation better than high-frequency songs, and transmission of short notes repeated at longer intervals is more efficient in dense vegetation (Farina, 2014; Morton, 1975). The song frequency in the wood doves is low and seems to support both MAH and AAH (Farina, 2014). Songs of smaller species have higher overall frequency, but all smaller species occur in less dense environments and are closer related. Hence it is hard to solve equivocally what was more important in shaping their song frequency: size, habitat, or phylogeny. First of all, wood doves have much lower song frequency than we can expect from a relationship found in birds and mammals and expressed by a power law $f \propto M^{-33}$ (where, f is frequency and M is body mass) (Fletcher, 2004; Wallschläger, 1980). Based on body masses of wood doves we should expect songs of frequencies between 2.05 and 2.51 kHz on average, while we found that average song frequency of these species varied from 0.44 to 0.59 kHz and was negatively correlated with body mass (r = -0.66). The abovementioned power law was formulated based on the analysis of 90 species of songbirds and confirmed for some mammals. Thus, it is possible that there might be different allometric relationships between signal frequency and body size in doves. Indeed, E. Morton compared *oscines*, *suboscines* and *nonpasseriformes* and found that phylogeny influences the size-frequency allometry (Morton, 1975). He also predicted that in forest habitat birds should sing with frequencies that are relatively low for their size and even their taxonomic unit. Furthermore, within relatives (here *Turtur* sp.), we should expect species inhabiting grassland or edge habitats to sing with higher frequencies. Our study supports these ideas very well. However, even among studied doves, we have some interesting exceptions. The song of *T. tympanistria* ($\bar{x}$ ± SD 443 ± 25 Hz) has very similar frequency as the song of the second forest wood dove, *T. brehmeri* (446 ± 21 Hz), while the first species is much smaller (68 g vs 112 g of body masses on an average). This is an interesting observation as we know that song frequency is very individually stable, on the one hand, and that males are taking song frequency into account during interactions (Niśkiewicz et al., 2024b; Osiejuk et al., 2019). As we know, there is also negative and significant within-species correlation between body size measurements and song frequency in some *Turtur* sp. (own unpublished material), hence, this song feature could be under strong sexual selection, which potentially is differentiated among species. Within the genus, *T. tympanistria* is singing much lower frequency songs than we should expect based on its size, while the other species seem to follow the same allometric rule. This finding contradicts the expectation of ANH, as this hypothesis predicts that species reduce competition for acoustic spectrum which in wood dove case spectrum of both forest and savannah species overlap to great extent (Farina, 2014). Indeed, our recent experiments indicate that smaller males of *T. tympanistria* tend to avoid responding to the songs of larger *T. brehmeri* iddividuals in sympatry, and are likely to spatially avoid them at the microhabitat scale (Niśkiewicz, Szymański, Budka, et al., 2023).

As mentioned earlier, the AAH predicts also differentiated temporal pattern of song components, in our model, note duration and pauses. Our study supports this hypothesis; differences in note duration and pauses seem to perfectly match this prediction as we compare *Turtur* species along their preferred habitat gradient. The differences between two forest and three savannah species are, of course, much smaller concerning these traits. We have examples of a similar relationship between habitat type and song structure. The little greenbul (*Eurillas virens*) population inhabiting forest and ecotones with divergent ambient noise spectra led to an association between morphological and acoustic divergence (Slabbekoorn & Smith, 2002). In the song sparrow (*Melospiza melodia*) analogical differences in song elements spacing were observed with habitat gradient in California (Patten et al., 2004). However, several recent studies questioned the strength of the relationship between habitat type and signal frequency, suggesting that habitat structure only weakly predicts the properties of bird songs (Boncoraglio & Saino, 2007; Ey & Fischer, 2009; Friis et al., 2021; Mikula et al., 2021). However, These studies compare large species and usually do not include detailed information about sound transmission in particular microhabitat and song behaviour, like song post location etc. (Mikula et al., 2021). Therefore, we posit that a critical component in assessing the predictions of the Acoustic Adaptation Hypothesis hinges on comparing small, closely related species with well-documented vocalisation patterns.

5.4. Species and individual recognition

The earlier discussion was focused on the general relationships between song features, birds' morphology, phylogeny and their habitat acoustics. We did not consider what type of information is transmitted with the song of wood doves, while consistent double-part song structure suggests that *Turtur* doves might have a two-level signalling strategy. The majority of better-studied pigeons and doves have entirely different songs than *Turtur* doves. They typically produce repeated cooing, which is relatively short and reproduced in repeated series without considerable variation (Beckers & ten Cate, 2001; de Kort et al., 2002). It seems that the strategy for attracting a mate or deterring a rival is, in such species, redundant signalling, which could be stopped freely when a signaller is not motivated. The phrase as a basic song unit produced by *Turtur* doves is much longer than a typical *Streptopelia* syllable, building a longer sequence of cooing. The clear two-part structure suggests that the signal evolved under pressure forcing the separation of information into a distinct part of the signal. Many evolutionary scenarios can be responsible for the evolution of such songs. Double-part songs quite often differ in a way, which results in the differentiation of the active ranges of song parts (Dabelsteen, 2004). Different mechanisms can achieve such differentiation. The most obvious is the differentiated amplitude of song parts; however, due to frequency-dependent differences in attenuation and distortion, the sounds of different frequencies also have propagation range consequences. Both mechanisms, amplitude and frequency, are often used together by signallers. Such phenomenon occurs in the blackcap (*Sylvia atricapilla*) (Linossier et al., 2015), in the gray-cheeked fulvetta (*Alcippe morrisonia*) (Shieh et al., 2013) or in some pipits, (*Anthus* spp.), (Elfström, 1990), where the quieter, whispered parts are aimed at nearby individuals and the louder ones carry information further away, beyond the contact range. However, probably the best-studied example is the European blackbird (*Turdus merula*) (Dabelsteen, 1981; Dabelsteen et al., 1993; Dabelsteen & Pedersen, 1988). The initial song part is quieter than the final in the studied wood doves. However, these differences are much more prominent in savannah species than forest ones. We observed differences in within-phrase relative amplitude differentiation and large differences in maximal song amplitudes between forest and savanna species; hence, the active ranges of songs in these two groups of wood doves dramatically differ. The potential functional consequences of these song phrase characteristics are that a large portion of the initial song part has a limited active range (Figure 30, Figure 31). In both forest species, the song's second half may reach receivers at quite a long distance (> 300 m). In the savannah species, the pattern is even more complicated. *T. abyssinicus* is very similar to forest species but has a lower level of song amplitude and, consequently, signal active range. In the remaining savannah wood doves, *T. chalcospilos* and *T. afer*, only a tiny portion of song phrases in the middle is loud and potentially reaches distant receivers. It is worth mentioning here that doves' hearing abilities are similar to those of human (Brand & Kellogg, 1939; Cook et al., 2016; Friedrich et al., 2007; Heise, 1953; Lewald, 1987). Thus, the direct comparison with humans is therefore legitimate. For example, the smallest wood dove, *T. abyssinicus*, could be detected by experienced observers from 150-250 m. However, to hear the initial part of the song as well, it is necessary to be as close as 10-20 m to the bird. Regardless of the actual song functions, they are likely related to these differentiated active ranges, as different parts of songs greatly vary in this respect.

Analyses we conducted in this study revealed that despite a general similarity of songs between *Turtur* doves, there are species- and -individual-specific variations. We found a general pattern indicating that the low-amplitude initial part of the song carries more individually specific information than the high-amplitude final part, which seems to be more informative about species. Such a separation of active ranges for different types of information encoded within songs seems to be functionally justified. Mate attraction and rival deterrence work efficiently if the signal has an extensive acting range. On the other hand, interaction with known neighbours or own mate could be more sophisticated and sometimes even lead to the evolution of quiet signals, in which limiting the active range is crucial, regardless of their ultimate functions (Jakubowska & Osiejuk, 2018; Osiejuk, 2011; Ręk & Osiejuk, 2011). In the case of wood doves, this role should be fulfilled by the first, initiating part of the song. While the final, more homogeneous, louder and lower-frequency part may carry further distances, so it should carry information about the sender's species (Mathevon et al., 2005). Such differences may also indicate that the signal is intended for a specific audience (Dabelsteen and McGregor, 1996), i.e. quiet identity information for local recognition and loud species information for distant receivers. Some tropical bird species living in visually occluded habitats evolved functionally sophisticated songs, by meaning of transmitting identity information through environment and optimal range. For example, the white-browed warbler (*Basileuterus leucoblepharus*) from the Brazylian Atlantic forest, has quite similar songs as *Turtur* doves, but with decreasing frequency, allowing for limiting individual information to less than 100 m in the forest (Aubin et al., 2004). We suppose that wood dove songs may have similar functionally but achieved due to different mechanism: differentiation of amplitude. Testing discrimination and ranging issues of their songs together will be crucial to include in further studies. However, answering detailed questions about coding identity (both species and individual) and signal range in the field would demand sophisticated experimental design. Currently, our knowledge in this respect is very limited. From experiments conducted on *T. brehmeri* and *T. tympanistria* in allopatry and sympatry, we may conclude that wood doves probably learn to respond to the proper (own species) signal during active interactions in early life (Niśkiewicz, Szymański, Budka, et al., 2023). At least, *T.*

tympanistria males who never had contact with *T. brehmeri* song approach the speaker with playback of congener's songs. This study indicates that, regardless of the lack of song production learning in Columbidae, the acoustic contests might be dynamic in time and space and related to the presence of related competitors. For example, *T. chalcospilos* were found to respond strongly only to their own species song in sympatry with *T. afer*, hence, similarly to what was observed in *T. brehmeri* and *T. tympanistria* in sympatry (Niśkiewicz, Szymański, Budka, et al., 2023; Niśkiewicz, Szymański, Zampa, et al., 2023). Moreover, the pattern of responding to mixed songs, artificially built of initial and final phrases of both *T. chalcospilos* and *T. afer*, does not support the idea that only a particular part of the song codes belong to a species. These two experiments instead suggest that the lower amplitude part of the songs is related to the limiting range of identity while the species-specific information is embedded in the entire song but with different strengths. However, as we found pretty substantial differences between different wood dove species, this should be more thoroughly tested. One of the experiments with *T. brehmeri* indicates that males of this species can discriminate between neighbours and strangers and respond vigorously to higher potential threats. Moreover, responding males likely extract information on body size from the song pitch; however, it should be demonstrated in a more straightforward experiment (Niśkiewicz et al., 2024b). Without doubt, wood doves from genus *Turtur*, due to their specific song structure and common occurrence in preferred habitats, are perfect model for studying how information is coded and transmitted through differentiated environment. Previous research on the encoding of identity or species information in vocal signals rarely concerned pigeons and doves, leaving a significant gap in our understanding this family. This study addresses this gap, shedding light on the intricate communication patterns and species recognition mechanisms of doves.

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Supplement 1. Photographs of the studied *Turtur* doves.

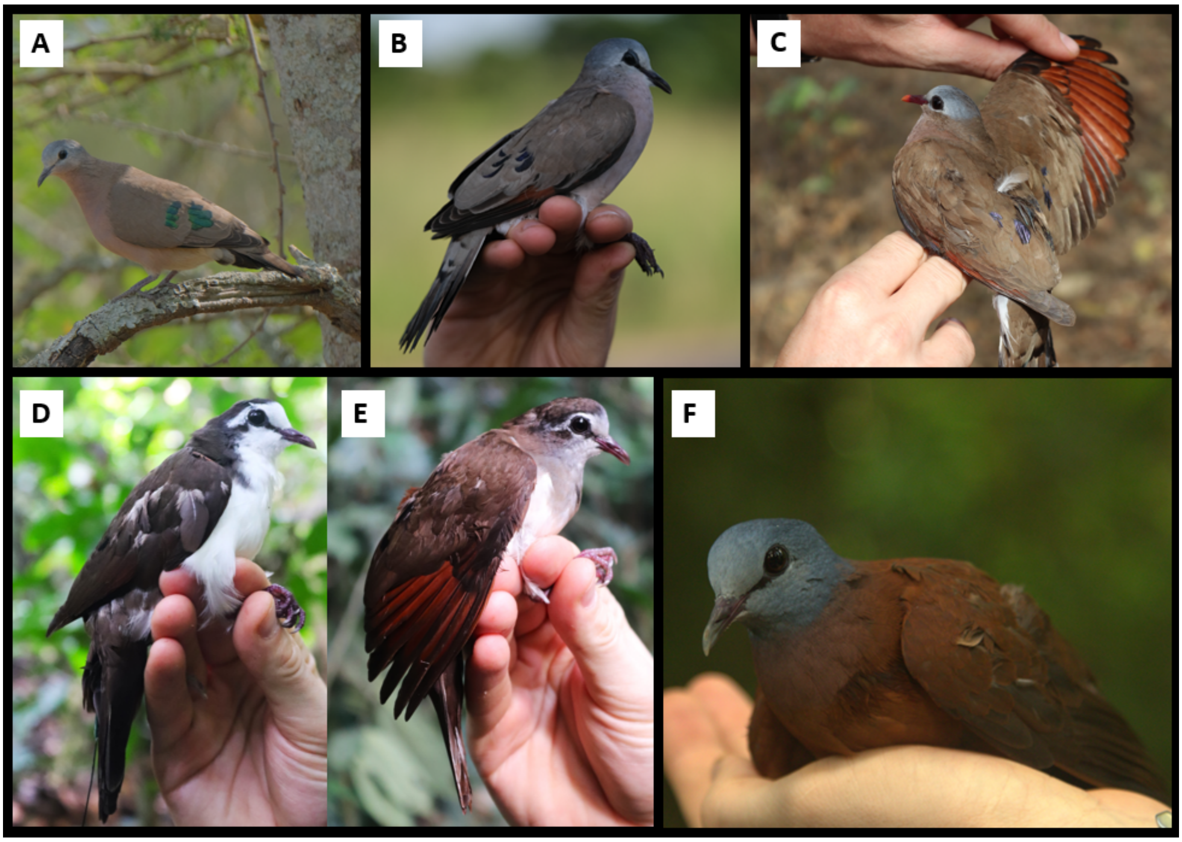

Figure S 1. Photos of all members of the *Turtur* genus: A – *T. chalcospilos*, B – *T. abyssinicus*, C – *T. afer*, D – *T. tympanistria* (male), E – *T. tympanistria* (female), F – *T. brehmeri*.

Supplement 2. Body measurements of *Turtur* doves.

Table S 1. Wing length of the *Turtur* doves.

Species	$\bar{x}$ ± SD (mm)	Range (mm)	N
T. brehmeri	135 ± 6.4	124-144	11
T. tympanistria	113 ± 3.9	106-121	50
T. afer	111 ± 4.7	98-121	34
T. chalcospilos	111 ± 2.2	108-116	32
T. abyssinicus	107 ± 2.6	101-112	33

Table S 2. Tail length of the *Turtur* doves.

Species	$\bar{x}$ ± SD (mm)	Range (mm)	N
T. brehmeri	115 ± 4.6	104-120	11
T. tympanistria	91 ± 6.4	77-106	50
T. afer	92 ± 7.6	80.5-111	33
T. chalcospilos	79 ± 2.7	72-85	32
T. abyssinicus	85 ± 3.9	72-92	33

Table S 3. Tarsus length of the *Turtur* doves.

Species	$\bar{x}$ ± SD (mm)	Range (mm)	N
T. brehmeri	25.8 ± 1.54	21.55-27	11
T. tympanistria	22.9 ± 1.97	18.6-26	50
T. afer	20.8 ± 1.91	17.8-25	34
T. chalcospilos	19.1 ± 0.82	17-20.85	32
T. abyssinicus	18.7 ± 0.75	17.5-20.15	33

Table S 4. Beak length of the *Turtur* doves.

Species	$\bar{x}$ ± SD (mm)	Range (mm)	N
T. brehmeri	17.2 ± 0.51	16-17.85	11
T. tympanistria	15.3 ± 0.98	13.2-18.2	50
T. afer	14.7 ± 0.96	12.1-16.15	34
T. chalcospilos	14.3 ± 0.88	12.15-16.4	32
T. abyssinicus	14.6 ± 0.56	13.65-14.65	33

Table S 5. Beak height of the *Turtur* doves.

Species	$\bar{x}$ ± SD (mm)	Range (mm)	N
T. brehmeri	5.6 ± 0.32	5-6.1	11
T. tympanistria	4.5 ± 0.43	3.65-5.6	50
T. afer	4.1 ± 0.29	3.65-4.8	34
T. chalcospilos	3.9 ± 0.24	3.25-4.5	32
T. abyssinicus	3.7 ± 0.27	3.1-4.3	33

Table S 6. Head with beak length of the *Turtur* doves.

Species	$\bar{x}$ ± SD (mm)	Range (mm)	N
T. brehmeri	44.2 ± 1.19	42.25-46.2	11
T. tympanistria	38.2 ± 1.00	35.6-40.2	50
T. afer	37.8 ± 1.05	35.1-40.2	34
T. chalcospilos	37.4 ± 0.92	35.65-39.4	32
T. abyssinicus	37.6 ± 0.93	34.5-39.25	33

Table S 7. Body mass of the *Turtur* doves.

Species	$\bar{x}$ ± SD (g)	Range (g)	N
T. brehmeri	113 ± 7.9	98.5-129.5	11
T. tympanistria	68 ± 6.6	51.5-87	50
T. afer	63 ± 5.7	49.5-73	34
T. chalcospilos	63 ± 4.2	57-75	30
T. abyssinicus	61 ± 4.5	52.5-73	33

Supplement 3. Details of body size comparisons – tests.

Table S 8. Results of ANOVAs (F-statistics and P values given) conducted to compare species differences in body size measurements with Bonferroni corrected paired comparisons.

Description of abbreviations: TB – *Turtur brehmeri*, TT – *Turtur tympanistria*, TAf – *Turtur afer*, TC – *Turtur chalcospilos*, TAb – *Turtur abyssinicus*
Significance of paired comparison: ns $P > 0.1$; • $P = 0.01\text{-}0.05$; * $P = 0.05\text{-}0.01$; ** $P = 0.01\text{-}0.001$; *** $P < 0.001$

Measurement: Wing

$F = 113.67$. $P < 0.0001$

	TB	TT	TAf	TC
TT	***			
TAf	***	ns		
TC	***	ns	ns	
TAb	***	***	***	***

Measurement: Tail

$F = 94.23$, $P < 0.0001$

	TB	TT	TAf	TC
TT	***			
TAf	***	ns		
TC	***	***	***	
TAb	***	***	***	**

Measurement: Tarsus

$F = 73.08$, $P < 0.0001$

	TB	TT	TAf	TC
TT	***			
TAf	***	***		
TC	***	***	***	
TAb	***	***	***	ns

Measurement: Beak length

$F = 28.43$, $P < 0.0001$

	TB	TT	TAf	TC
TT	***			
TAf	***	*		
TC	***	***	ns	
TAb	***	**	ns	ns

Measurement: Beak height

$F = 82.01$, $P < 0.0001$

	TB	TT	TAf	TC
TT	***			
TAf	***	***		
TC	***	***	•	
TAb	***	***	***	ns

Measurement: Head with beak length

$F = 108.48$, $P < 0.0001$

	TB	TT	TAf	TC
TT	***			
TAf	***	ns		
TC	***	**	ns	
TAb	***	*	ns	ns

Measurement: Body mass

$F = 196.69$, $P < 0.0001$

	TB	TT	TAf	TC
TT	***			
TAf	***	***		
TC	***	**	ns	
TAb	***	***	ns	ns

Supplement 4. Differences in habitat preferences of *Turtur* doves revealed by meaning of NDVI and EVI comparison.

Table S 9. Results of Gaussian mixed model (identity link) with NDVI as response variable and species and geographical coordinates as predictors. We also included a random intercept hemisphere and date in the model. Number of observations = 9144, number of groups: hemisphere = 2; date = 23.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	0.633	0.015	42.98	< 0.001
Species	-0.021	0.002	-12.70	< 0.001
Latitude	0.000349	0.0002162	1.61	0.106
Longitude	0.0017	0.00012	14.72	< 0.001
Random effects				
Groups	Name	Variance	Std. Dev.	
Hemisphere	Intercept	1.036e-11	3.218e-06	
Date	Intercept	4.158e-03	6.448e-02	

Table S 10. Results of Gaussian mixed model with EVI as response variable and species and geographical coordinates as predictors. We also included a random intercept hemisphere and date in the model. Number of observations = 9144, number of groups: hemisphere = 2; date = 23.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	0.4390	0.0122	36.08	< 0.001
Species	-0.0135	0.0013	-10.18	< 0.001
Latitude	-0.0026	0.00018	-14.62	< 0.001
Longitude	0.00010	0.00014	0.72	0.473
Random effects				
Groups	Name	Variance	Std. Dev.	
Hemisphere	Intercept	5.943e-05	0.0077	
Date	Intercept	2.095e-02	0.1447	

Supplement 5. Summaries of GLMM models examining the effect of species (5 wood dove species) and part of the song (initial and final) on characteristics of elementary song unites (notes and their derivatives).

Table S 11. Results of mixed model with a number of notes as the response variable and species and part of the song as predictors. We included song phrases nested in individuals as random factors.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	-18.81	1.255	-14.98	< 0.001
Species	9.25	0.380	24.30	< 0.001
Part of song	20.87	0.340	61.27	< 0.001
Species × Part of song	-5.87	0.115	-52.25	< 0.001

Table S 12. Results of a mixed model with a note duration (square root transformed) as the response variable and species and part of the song as predictors. We included song phrases nested in individuals as random factors. Statistically significant results are highlighted in bold.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	0.53	0.005	100.20	< 0.001
Species	-0.003	0.0016	1.685	0.0902
Part of song	-0.135	0.003	-45.05	< 0.001
Species × Part of song	0.017	0.0009	18.034	< 0.001

Table S 13. Results of a mixed model with a pause duration (square root transformed) as the response variable and species and part of the song as predictors. We included song phrases nested in individuals as random factors. Statistically significant results are highlighted in bold.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	1.95	0.014	143.14	< 0.001
Species	-0.26	0.004	-62.93	< 0.001
Part of song	-0.80	0.008	-102.70	< 0.001
Species × Part of song	0.14	0.002	53.56	< 0.001

Table S 14. Results of a mixed model with a pulse-to-pulse intervals (PTP, square root transformed) as the response variable and species and part of the song as predictors. We included song phrases nested in individuals as random factors. Statistically significant results are highlighted.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	1.99	0.013	153.53	< 0.001
Species	-0.23	0.004	-58.06	< 0.001
Part of song	-0.77	0.007	-104.66	< 0.001
Species × Part of song	0.13	0.002	53.43	< 0.001

Table S 15. Results of a mixed model with a rhythm (log-transformed) as the response variable and species and part of the song as predictors. We included song phrases nested in individuals as random factors. Statistically significant results are highlighted in bold.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	0.20	0.002	91.16	< 0.001
Species	-0.006	0.0006	-9.67	< 0.001
Part of song	-0.007	0.001	05.892	< 0.001
Species × Part of song	0.003	0.0004	7.45	< 0.001

Table S 16. Results of a mixed model with a note peak frequency (log-transformed) as the response variable and species and part of the song as predictors. We included song phrases nested in individuals as random factors. Statistically significant results are highlighted in bold.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	2.64	0.002	1361.83	< 0.001
Species	0.042	0.0006	72.17	< 0.001
Part of song	-0.016	0.0011	-14.70	< 0.001
Species × Part of song	-0.006	0.0003	-17.59	< 0.001

Table S 17. Results of Gaussian mixed model with a relative amplitude (log-transformed absolute value of relative amplitude) as the response variable and species and part of the song as predictors. We included song phrases nested in individuals as random factors. Statistically significant results are highlighted in bold.

Fixed effects	Estimate	Std. Error	*z* value	*P* value
Intercept	-0.58	0.018	31.61	< 0.001
Species	0.22	0.006	38.90	< 0.001
Part of song	0.001	0.01	0.099	0.921
Species × Part of song	-0.13	0.003	-37.07	< 0.001

Supplement 6. Potential for identity coding in the entire song phrase characteristics of all *Turtur* doves.

Table S 18. Potential for identity coding in the entire song phrase characteristics of *T. brehmeri*.

Song phrase characteristics	Hs	DS
No of notes	0.08	0.32
No of notes in Initial and Final part	0.08	0.62
Duration	0.2	0.37
Frequency (min, avg, max)	1.04	0.68
All characteristics	1.71	0.91

Table S 19. Potential for identity coding in the entire song phrase characteristics of *T. tympanistria*.

Song phrase characteristics	H_s	*DS*
No of notes	-0.02	0.46
No of notes in Initial and Final part	0.51	0.64
Duration	0.42	0.36
Frequency (min, avg, max)	2.22	0.87
All characteristics	3.47	0.93

Table S 20. Potential for identity coding in the entire song phrase characteristics of *T. afer*.

Song phrase characteristics	H_s	*DS*
No of notes	0.15	0.41
No of notes in Initial and Final part	0.08	0.54
Duration	0.76	0.31
Frequency (min, avg, max)	1.01	0.80
All characteristics	1.12	0.92

Table S 21.Potential for identity coding in the entire song phrase characteristics of *T. chalcospilos*.

Song phrase characteristics	Hs	DS
No of notes	0.89	0.49
No of notes in Initial and Final part	1.3	0.76
Duration	1.39	0.54
Frequency (min, avg, max)	2.04	0.79
All characteristics	3.07	0.92

Table S 22. Potential for identity coding in the entire song phrase characteristics of *T. abyssinicus*.

Song phrase characteristics	H_s	*DS*
No of notes	0.87	0.41
No of notes in Initial and Final part	1.45	0.71
Duration	0.63	0.67
Frequency (min, avg, max)	0.66	0.78
All characteristics	2.64	0.98

Supplement 7. Spectrograms of different individuals of all *Turtur* dove species.

Spectrogram prepared with ‘warbleR’, ‘seewave’, ‘tuneR’ packages of R CRAN and the following set of parameters:
color.spectro(wave = file1, wl = 2048, wn = "hanning", ovlp = 90, flim = c(0,1),collevels = seq(-30,0,1), base.col = "blue", axisX = FALSE, axisY = FALSE, dB = "max0", flab = "")
Different bas.col colours used to indicate different individuals.

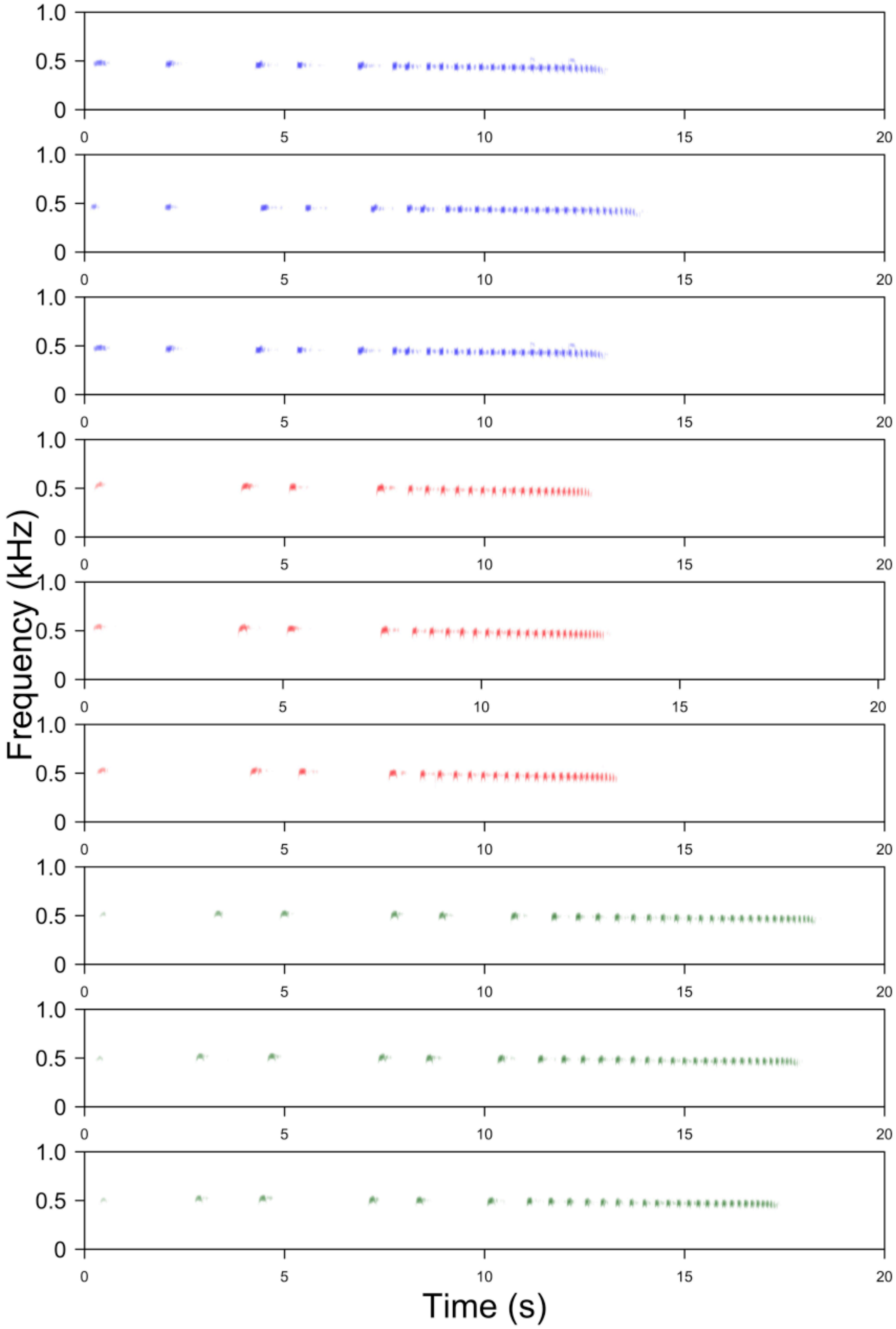

Figure S 2. Spectrograms illustrating songs of three different *Turtur brehmeri* males (colours indicate different individuals for easier comparison of individual differences).

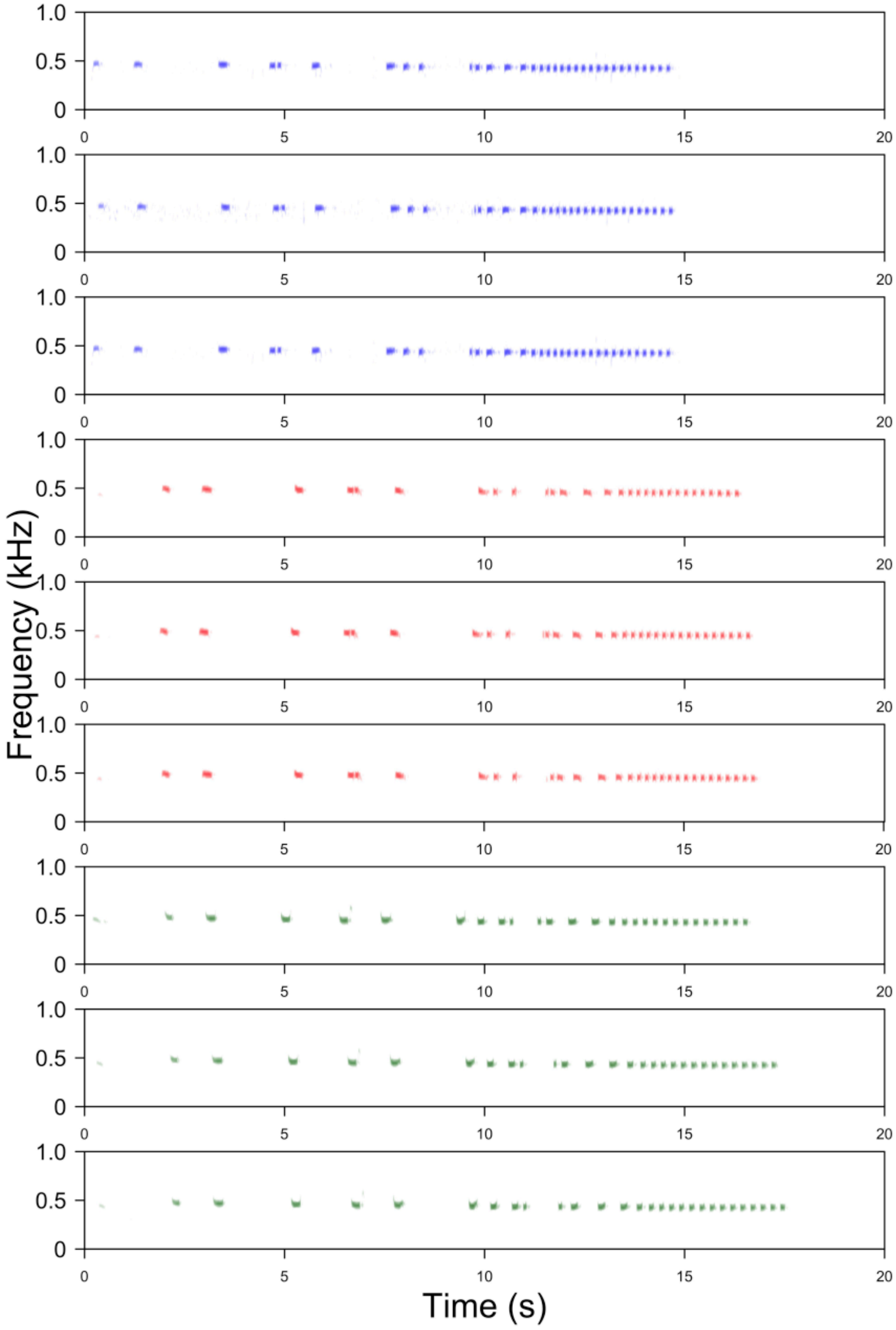

Figure S 3. Spectrograms illustrating songs of three different *Turtur tympanistria* males (colours indicate different individuals for easier comparison of individual differences).

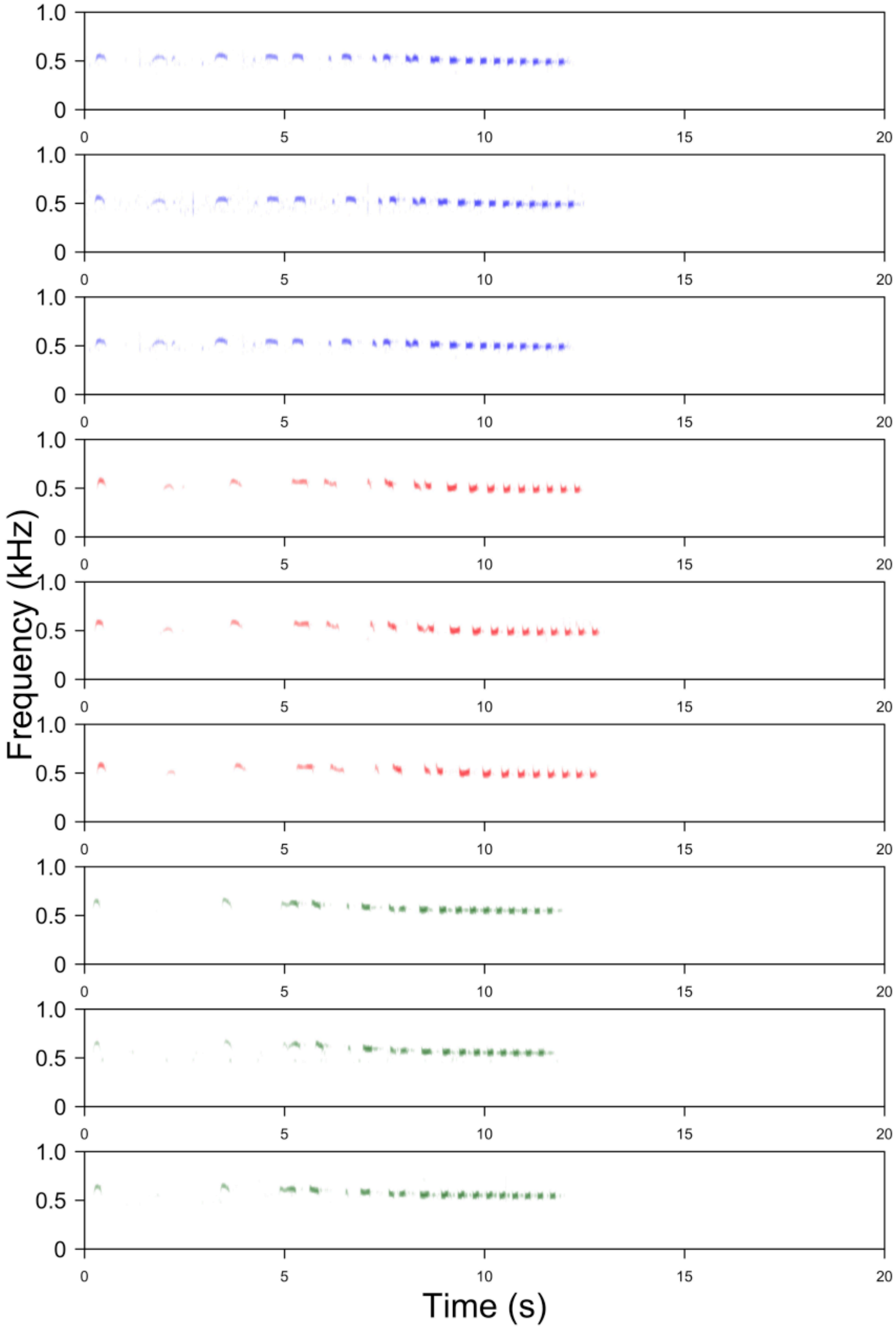

Figure S 4. Spectrograms illustrating songs of three different *Turtur afer* males (colours indicate different individuals for easier comparison of individual differences).

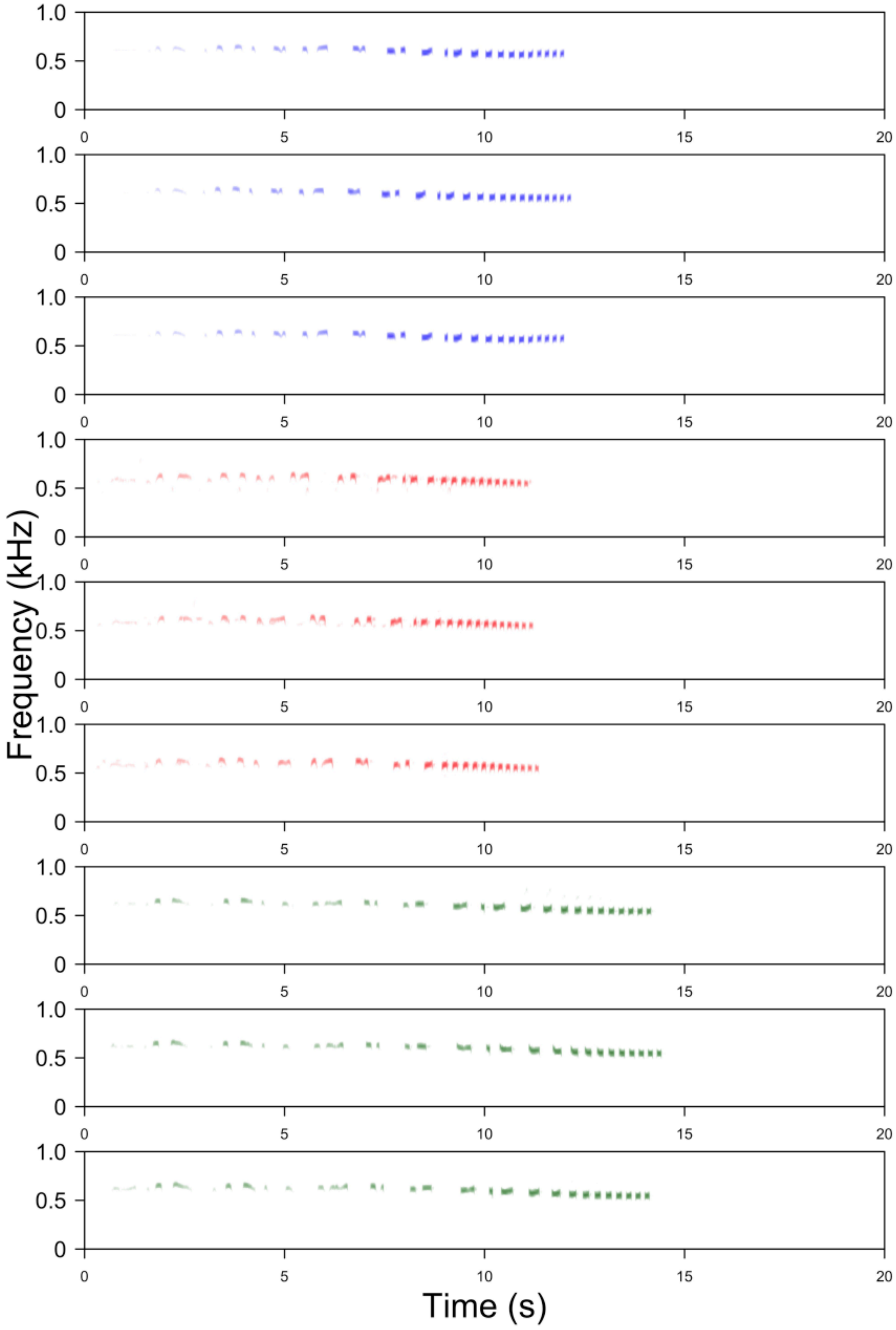

Figure S 5. Spectrograms illustrating songs of three different *Turtur chalcospilos* males (colours indicate different individuals for easier comparison of individual differences).

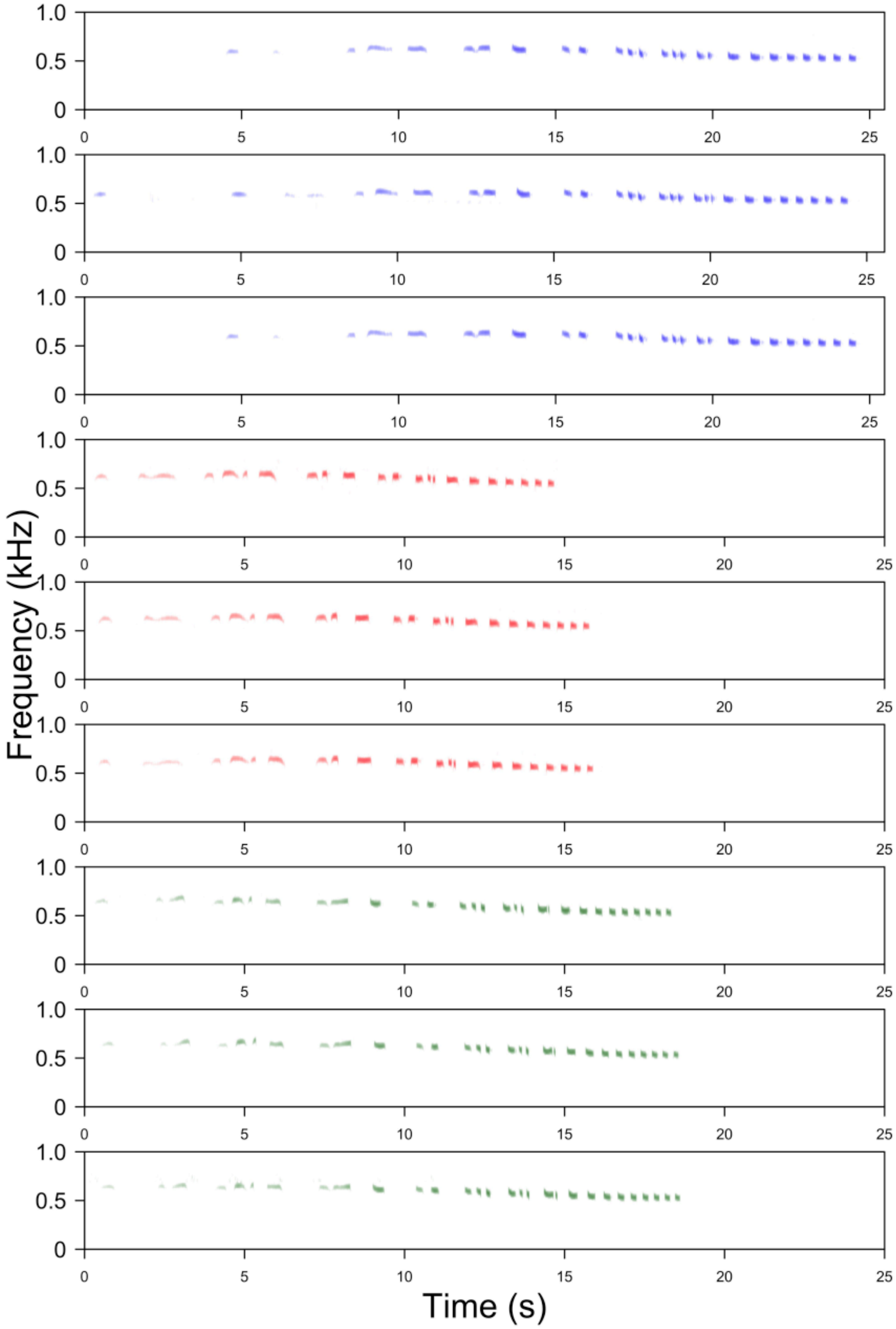

Figure S 6. Spectrograms illustrating songs of three different *Turtur abyssinicus* males (colours indicate different individuals for easier comparison of individual differences).

CHAPTER 2

Response of forest *Turtur* doves to conspecific and congeneric songs in sympatry and allopatry

Małgorzata Niśkiewicz, Paweł Szymański, Michał Budka
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Response of forest *Turtur* doves to conspecific and congeneric songs in sympatry and allopatry

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Birds have a diverse acoustic communication system, and the ability to recognise their own species' song from a distance facilitates complex behaviours related to mate attraction and rival deterrence. However, certain species, including doves, do not learn songs and their vocal repertoires are much simpler than those of better-studied songbirds. In these so-called non-learning birds, relatively little is known about the role that bird song plays in intra- and interspecific interactions, and how such behaviours might be acquired (inherited or learned from experience). To investigate this question, we focused on two species of African wood doves whose long-range songs are used in a territorial context. Specifically, we examined the responses of sympatric and allopatric populations of male blue-headed wood-doves (*Turtur brehmeri*) and tambourine doves (*Turtur tympanistria*) to different types of simulated territorial intrusions, i.e. playback of conspecific, congeneric, and control songs. We aimed to assess (i) whether these species, which have similar songs, respond only to their own species' song or exhibit interspecific territoriality, and (ii) if the response pattern is affected by the presence or absence of congeners in the general area. We found that both species responded strongly to playback of their own species in both sympatric and allopatric populations. In allopatry, though, male tambourine doves misdirected their response and also approached the playback of congeneric songs. Our results indicate that, in areas where the studied *Turtur* doves live in sympatry, they do not exhibit consistent interspecific territoriality. However, we cannot exclude the possibility that the smaller tambourine dove avoids its larger congener during the process of territory establishment. The difference in tambourine doves' response toward the song of present (sympatric) or absent (allopatric) congeners suggests that the ability to discriminate between songs of similarly singing potential competitors is acquired through earlier interactions and learning. This plasticity in response supports the misdirected aggression hypothesis, which argues that interspecific territorialism emerges as a maladaptive by-product of signal similarity. However, on an evolutionary timescale, such an ability could be considered an adaptive cognitive tool useful for resolving competing interests with congeners.

Acoustic communication is widespread in many animal taxa, but the evolution of complex sound signals has occurred in only a few groups. One such group is birds, whose songs exhibit an extraordinary degree of diversity and sophistication[1,2]. Apart from some basic pre-adaptations for vocal communication found in the ancestors of modern birds (e.g., breathing apparatus), the main factor that led to the evolution of elaborate songs was sexual selection. To wit, the most important functions of bird song are attracting a breeding partner and defending territory or other resources[2]. These two functions are not mutually exclusive and were historically assigned to males, as males were thought to be the dominant singing sex[3]. To fulfil these functions, songs must be species-specific as well as sufficiently individually unique to enable discrimination or recognition of individuals or classes of individuals (e.g., stranger vs neighbour)[2,4]. This two-level specificity of the song is then interpreted by the receiver for use in decision making, even in the absence of visual information[5].

In species where only the male sings, the meaning of a song could be different from the perspectives of male and female receivers. When a female recognises the song of her own species, she can, in the next step, evaluate the quality (or other features like dialect origin) of the sender and decide whether to reproduce with him[6,7]. A male receiver, on the other hand, is usually focused on extracting information about the threat level expressed by the signal. For example, when territory boundaries are established, the song of a neighbour singing from his territory border is usually treated as less dangerous than the song of a stranger[8]. The evaluation of a potential

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rival's song enables the territory owner to make an appropriate decision about interacting with that rival[9,10]. Responding too aggressively may lead to a risk of injury or death in the worst case or wasted energy in the best case[10,11]. For the territory owner and potential mate, a long-distance signal carrying a message about species and individual identity is usually crucial and a prerequisite for territorial behaviour[2].

Interspecific territoriality

Territoriality is widespread among animals[12–15]. Within a species, individuals prefer similar habitats and must compete for limited resources, but the exact pattern and level of aggressiveness exhibited during competition depend on their life histories (e.g., sedentary vs migratory lifestyle, breeding system)[16,17]. Initially, between-species interactions were assumed to be rare, costly, and the result of ancestral signal similarity. Surprisingly, recent comparative studies have revealed that interspecific territoriality is common in birds[18,19]. To explain the evolution of interspecies territorialism, four basic hypotheses have been proposed (reviewed in Cowen et al.[20]). The first is the resource competition hypothesis, which assumes that resources are partitioned spatially between dominant individuals of competing species[21–23]. The second hypothesis, asymmetric competition, is also based on strong resource rivalry but assumes that only dominant males of the dominant species succeed in interspecific territoriality[24]. The reproductive interference hypothesis explains the existence of territorial aggression between species as the result of competition not for the same resources but for a partner, due to incomplete reproductive isolation and the possibility of hybridisation[25,26]. The last hypothesis, misdirected aggression, posits that interspecies territorialism is maladaptive and a by-product of intraspecies territorialism[15,27].

These hypotheses reflect field observations and are not mutually exclusive. To shed light on this phenomenon, then, researchers must search for evolutionary scenarios in which these different options are possible, rather than try to eliminate erroneous hypotheses. Interspecific territoriality is more likely if species have somewhat similar songs and can physically interact[20]. Hence, the first step for understanding the process of such territoriality is examining if a species detects the signal of a congeneric and recognises it as worthy of a response. The hypotheses presented above provide some background for when, why, and how this might happen. As predominantly territorial animals, birds are particularly interesting models for studying interspecific territoriality because different groups of species are diametrically opposed to each other in the way acoustic signals are transmitted between generations (inherited vs learned). In addition, territorial defence in birds involves processing signals heard from a distance, and at least the initial part of the response is evoked solely by the sound signal[2].

Aims of the study

One interesting scenario for comparing potential interactions between species is when closely related species exploit similar resources and have similar signals. In such a case, interspecific interactions may be the result of competition, leading to interspecific territoriality and an intentional response to the other species (including avoidance of a 'stronger' competitor). However, interactions could also, at least potentially, be due to mistakes (similar signals). In addition, the character of such interactions might depend on whether the studied pair of species naturally occur in the same location (sympatry) or not (allopatry).

In this study, we experimentally tested the response of two non-learning, territorial bird species to conspecific and congeneric songs in both the sympatric and allopatric ranges. The main objectives were to see whether and how the studied species respond to each other's songs and how this response might change in areas in which the rival is not naturally found. Our models were the blue-headed wood-dove, *Turtur brehmeri* (hereafter *brehmeri*), and the tambourine dove, *T. tympanistria* (hereafter *tympanistria*). Genus *Turtur* contains a total of five species living in varied habitats of sub-Saharan Africa, and all have very similar songs that are used for long-distance communication. The songs consist of simple, low-frequency notes organised with generally similar syntax (songs have two parts differing in the duration of syllables, pauses, and peak frequency) and are quite different in comparison to other doves (Fig. 1). The model species both inhabit forests, but *brehmeri* has a range limited to central and western Africa, while *tympanistria* can be found in varied, forested habitats in almost all of sub-Saharan Africa[28,29].

In the area of sympatric occurrence, we tested both dove species. We expected birds to demonstrate a strong response to their own species' song. If the two species indeed compete in these habitats, the smaller species should avoid the larger intruder. For this reason, we predicted that *tympanistria* males would not respond to the congener's song or might even exhibit extreme avoidance behaviours (like flying away and ceasing to sing). Instead, the larger *brehmeri* should attempt to chase away its smaller competitor. Thus, we also expected that *brehmeri* males might respond to the *tympanistria* song, but not necessarily as strongly as to that of conspecific rivals. If the species do not exhibit interspecific territoriality, however, we would not expect to observe any response to the songs of congeners.

For our allopatric sites, we conducted experiments in a forest where only *tympanistria* occurs. Again, we expected a strong response to the conspecific song. Regarding the response to the absent congener, we investigated the possibility of two potential scenarios. Males of *tympanistria* may not respond to an unknown congener's song if it is not recognised as a potential competitor. Such a result would suggest that recognition of species-specific songs is inherited, and the relatively small differences between *tympanistria* and *brehmeri* songs facilitate efficient recognition even in the absence of any earlier experience with the congeneric species. However, if the allopatric *tympanistria* males respond to the song of a congener, it would support the misdirected aggression hypothesis, i.e., it is likely to be a case of mistaken identity due to the general similarity of both species' songs. It would also imply that any kind of response found between *tympanistria* and *brehmeri* in sympatry must result from earlier interactions between individuals of both species.

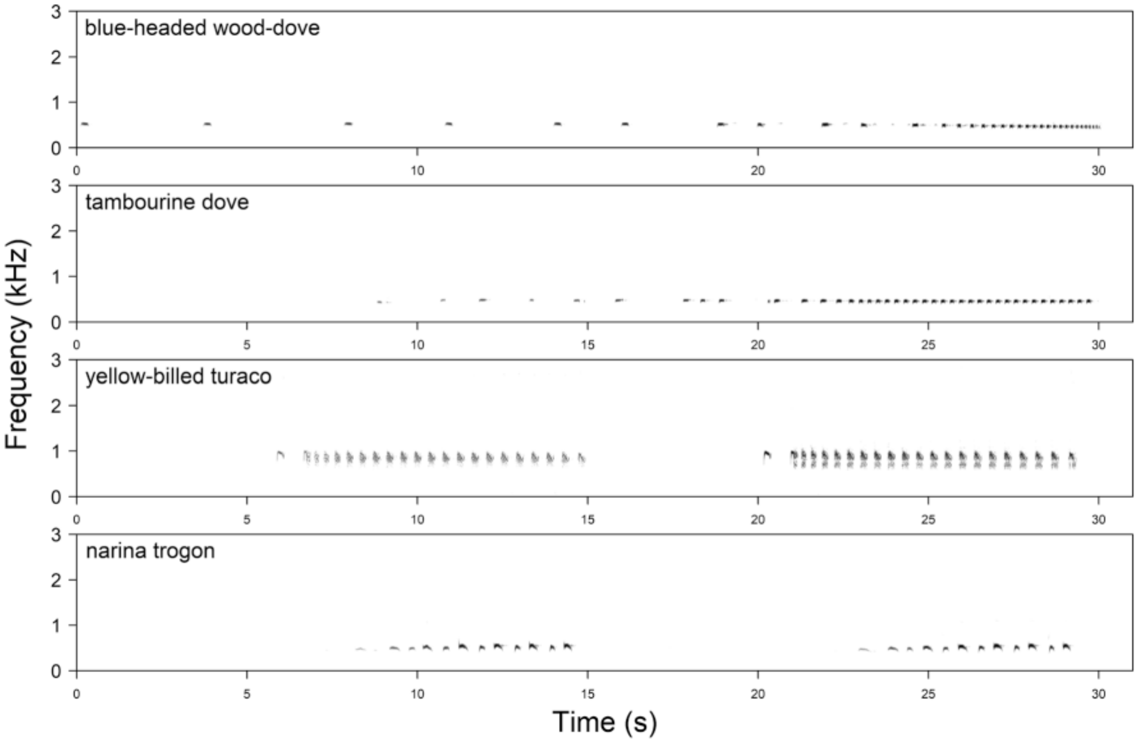

Figure 1. Spectrograms of the songs of focal bird species: blue-headed wood-dove (*Turtur brehmeri*) and tambourine dove (*Turtur tympanistria*), and species used as a control: yellow-billed touraco (*Tauraco macrorhynchus*) and narina trogon (*Apaloderma narina*).

Methods

Study sites and species

The study was conducted in two sites in sub-Saharan Africa, one where *brehmeri* and *tympanistria* occur in sympatry and one where only *tympanistria* is present. The first site was in Kakum National Park, in the coastal region of southern Ghana (5.20–5.40 N, 1.30–1.51 W, altitude 135–250 m asl). The park covers an area of ~ 210 km^2 of tropical moist evergreen forest with fragments of periodic or permanent swamp and riverine forests. Although logging operations between 1975 and 1989 decreased areas of dense primary forest, the logged areas have regenerated secondary forests characterised by denser vegetation. Kakum NP receives an annual average rainfall of 1460 mm, with most precipitation in September–October and March–June. Temperatures vary between 20 and 30 °C[30,31]. The second site was in Kibale National Park in Uganda (0.13–0.41 N, 30.19–30.32 E, altitude 1100–1600 m asl), a moist, evergreen, medium-altitude forest with a mosaic of primary and secondary forest, swamp, grassland, and thicket. Temperatures are very stable over the course of the year (daily fluctuation 15–27 °C) and the precipitation (annually around 760 mm) has a bimodal pattern, with more rainfall in March–April and September–November[32]. There appears to be no detectable bird seasonality in this area[33].

In this study, we focused on two *Turtur* species that prefer strictly forest-like habitats. The more ubiquitous *tympanistria* is typically found in any type of forest or forest-like habitat, such as lowland and montane forests (even up to 3000 m asl), secondary forests, gardens, plantations, or even mangroves[29]. The range of *brehmeri* is restricted to western Africa and lowland primary forests up to 750 m asl only. Occasionally it is also found in old secondary forests, but generally, it avoids heavily disturbed forests[28]. With a body mass of 98.5–129.5 g ($N = 10$), *brehmeri* are significantly ($t_{2,50} = 19.6$, $P < 0.0001$) larger than *tympanistria* (51.5–81 g; $N = 42$; own measurements in the study area). From our own observations and the literature, we found no evidence of hybridisation between these two species[34]. Furthermore, a STRUCTURE analysis performed on 7000 SNP markers (obtained by sequencing RADseq libraries) did not detect any signal of admixture between *brehmeri* and *tympanistria* (own unpublished data based on 6 *brehmeri* and 26 *tympanistria* individuals). We did not observe any direct, physical interaction between males of these species in the sympatric area, although both species were heard singing relatively close to each other. However, the above observations do not exclude the possibility of interspecific territoriality completely.

Playback stimuli

We prepared three types of playback: (i) conspecific (*brehmeri* and *tympanistria* for *brehmeri* and *tympanistria*, respectively), (ii) congeneric (*brehmeri* for *tympanistria* and *tympanistria* for *brehmeri*), and (iii) a control (yellow-billed tauraco, *Tauraco macrorhynchus*, in Kakum NP and narina trogon, *Apaloderma narina*, in Kibale NP). The control species were chosen because their territorial calls have similar spectra to the territorial songs of doves. Both species are common in the studied areas but are not perceived as a threat by doves and do not interact with them. To create playback stimuli of doves, we selected recordings of territorial songs of local non-neighbouring males with a high signal-to-noise ratio. Each song sample was filtered (high-pass, 0.1 kHz; low-pass, 1.5 kHz). Each playback stimulus was created from a sample belonging to a different individual to avoid pseudo-replication. Dove playback consisted of a single song sample repeated 10 times (two songs per minute), which is the typical calling rate of both *brehmeri* and *tympanistria*. Comparable song durations and pacing were also maintained for the control treatments. The amplitude of each playback was set to 82 ± 2 dB SPL at a 1-m distance from the speaker, measured with a CHY 650 digital sound level meter (CHY Firemate Co., Ningbo, China). All playback stimuli were created with Raven Pro 1.6 (Cornell Lab of Ornithology, Ithaca, USA) and Avisoft SASLab Pro 5.2 (Raimund Specht, Berlin).

Playback experiment design and procedures

General procedures of experiments I–III

Each male was tested three times (conspecific, congeneric, and control species treatments) in a counterbalanced order. Experiments were only conducted when a male was observed and heard singing before the playback. In each trial with a given male, the speaker was placed in a slightly different location to avoid the habituation of the focal bird, but always within the same distance (~ 50 m) of the place from which the bird was singing before playback and at the same height above the ground, ca. 2 m. To broadcast the playback stimuli, we used either a Tascam DR-40X (TEAC Europe GmbH, Wiesebaden, Germany) or Sony PCM-D100, and JBL Charge 4 speakers (Harman International Industries, Stamford, Connecticut, USA). Before starting the 5-min playback, we recorded the focal male for at least 1 min. Recorded songs were then used for checking if the responding individuals were the same as before the start of playback. The identity check was based on comparing the time and frequency distribution of the initial syllables in songs (see Fig. S1). This method was developed for *tympanistria* and allows for even 96% classification efficacy, depending on the number of compared syllables[35]. Analogous measurements allow for differentiating between males of *brehmeri* at a similar level (unpublished own data). Playback was then followed by a 5-min post-playback phase.

Each trial was observed by two people positioned on opposite sides of the speaker, ca. 20 m away from it, in locations that minimised the possibility of disturbing a bird approaching the speaker. The person recording songs had a lavalier microphone connected to the second channel of the main recorder for dictating observations. The second observer was on the opposite side of the speaker in a place convenient for observing the whole experimental scene. The birds' behavioural responses were recorded by dictating observations into the additional recorder. Recordings of vocalisations and comments on physical behaviour were later time-aligned and combined into one audio file. All distances reported (as the closest distances to the speaker) were measured with a Leica DISTO D510 laser range finder.

Our preliminary work with the study species revealed they are shy and that observing their behaviour in the forest is difficult. Therefore, we were focused a priori on those aspects of behaviour that we were able to quantify with sufficient and repeatable certainty among all experiments. In the case of the vocal response, we used the number of songs sung by males. The physical behavioural responses of males were measured as the time spent within 25 m of the speaker (s), latency to approach the speaker (s), the closest approach to the speaker (m), and the number of flights.

Experiments in sympatric area

Playback for Experiment I (with *brehmeri*) and Experiment II (with *tympanistria*) was performed between November 12 and December 1 in 2021 between 6:04 and 12:43 local time (sunrise started 5:52–5:58) in Kakum NP. All males were tested three times, with the three types of songs (conspecific, congeneric, and control song of yellow-billed turaco) presented in a counterbalanced order. The time of testing reflected birds' activity and depended on the weather on a particular day (in practice, single trials were performed after 12:00 on two separate occasions when we waited longer for birds after two earlier tests). Altogether, we tested 19 males of *brehmeri* and 14 males of *tympanistria*. The average time between the following treatments with the same male was 32 min for Experiment I and 39 min for Experiment II.

Experiment in allopatric area

Playback for Experiment III was performed between June 28 and July 6 in 2022. We tested birds during their active time in the morning, 6:58–9:47 (sunrise started between 5:59 and 6:00), and in the late afternoon, 16:44–17:48 (sunset started between 18:03 and 18:05). Each male was tested only in the morning or the evening, as their pattern of activity was strictly bimodal and short due to weather conditions. Altogether, in Kibale NP we tested 15 males of *tympanistria*. The three songs of each trial were presented in a counterbalanced order, exactly as in Experiments I-II, and differed only in the species used for the control (narina trogon). The average time between subsequent treatments with the same male was 21 min.

Ethical note

To our knowledge, the individuals tested in the experiment reflected the population in a representative way with no potential biases resulting from social background, self-selection, habituation, or other factors as indicated in the STRANGE framework (Webster and Rutz 2020). This study was designed and performed in accordance with the ARRIVE guidelines[36]. Our experimental procedure adhered to the ASAB/ABS Guidelines for the care and use of animals (The Ethics Committee (ASAB) and the Animal Care Committee (ABS), 2019) and was approved by all responsible local bodies listed below as well as by the Polish Laboratory Animal Science Association (certificate no. 1952/2015 to TSO, conforming to Directive 2010/63/EU). Our experimental procedures were approved in Ghana by the Forestry Commission (Wildlife Division), permit no. WD/A.185/Vol.13/80, and in Uganda by

Makerere University (College of Health Sciences; Makerere University Biological Field Station) and the Uganda Wildlife Authority, permit no. COD/96/05 and Research Material Transfer MTA no. 377, as well as the Uganda National Council for Science and Technology, permit no. NS256ES.

Statistical analysis

The original response variables we measured during experiments were partly correlated with each other, but the multicollinearity was moderate (variance inflation ratio VIF between 1.02 and 3.42). Therefore, to analyse the general strength of the response to playback, we used a principal component analysis (PCA) with varimax rotation and Kaiser normalisation (IBM SPSS Statistics 28.0.1.0). We extracted principal components separately for all three experiments. All three datasets were well suited for PCA (KMO and Bartlett's tests are given in Tables 1, 2, 3); the first two extracted components explained a similar percentage of the variance (PC1: 43.77–47.93% and PC2: 25.52–30.40%) and had a similar pattern of loadings from the original variables. All the first components

Statistics and original response variables	PC1—approaching	PC2—vocal response
Eigenvalue	2.936	1.515
% of variance	43.773	30.401
Cumulative %	43.773	74.173
Songs during playback	– 0.26	**0.88**
Songs after playback	0.01	**0.92**
Flights during playback	**0.80**	– 0.26
Flights after playback	0.66	0.26
Closest distance (m)	**– 0.89**	0.22
Time < 25 m to speaker (s)	**0.83**	– 0.16

Table 1. Principal component loadings for blue-headed wood-dove responses to playback in sympatry (Experiment I). Kaiser–Meier–Olkin = 0.702, Bartlett's test of sphericity $\chi^2 = 150.245$, $P < 0.001$. Values that make a substantial contribution to the overall variance are in bold.

Statistics and original response variables	PC1—approaching	PC2—vocal response
Eigenvalue	2.693	1.636
% of variance	44.879	27.275
Cumulative %	44.879	72.153
Songs during playback	– 0.24	**0.85**
Songs after playback	– 0.03	**0.80**
Flights during playback	**0.95**	– 0.04
Flights after playback	0.29	0.53
Closest distance (m)	**– 0.89**	0.03
Time < 25 m to speaker (s)	**0.91**	0.05

Table 2. Principal component loadings for tambourine dove responses to playback in sympatry (Experiment II). Kaiser–Meier–Olkin = 0.656, Bartlett's test of sphericity $\chi^2 = 113.173$, $P < 0.001$. Values that make a substantial contribution to the overall variance are in bold.

Statistics and original response variables	PC1—approaching	PC2—vocal response
Eigenvalue	2.876	1.531
% of variance	47.926	25.522
Cumulative %	47.926	73.447
Songs during playback	– 0.13	**0.91**
Songs after playback	0.30	**0.84**
Flights during playback	**0.92**	– 0.08
Flights after playback	0.53	0.13
Closest distance (m)	**– 0.93**	– 0.07
Time < 25 m to speaker (s)	**0.85**	0.07

Table 3. Principal component loadings for tambourine dove responses to playback in allopatry (Experiment III). Kaiser–Meier–Olkin = 0.675, Bartlett's test of sphericity $\chi^2 = 123.563$, $P < 0.001$. Values that make a substantial contribution to the overall variance are in bold.

had heavier loadings from variables related to the approach to the speaker (Tables 1, 2, 3), while all the second components had heavier loadings from the variables related to singing. Therefore, we refer hereafter to PC1 as the 'Approaching' component and to PC2 as the 'Vocal response' component (Tables 1, 2, 3). Higher values of PC1 indicate more flights during the playback, a closer approach to the speaker, and more time spent in its vicinity, hence, a stronger response. Higher values of PC2 indicate that responding birds sang more songs both during and after playback.

To test for differences in the response to conspecific, congeneric, and control songs, we built generalised linear mixed-effects models (GLMM) using the 'lme4' package of R[37] and checked model assumptions using the DHARMa package[38]. Our response variables were the measures of approaching (PC1) and vocal response (PC2) extracted separately for each experiment. We included in our models two main factors: (1) playback treatment (three levels: conspecific, congeneric, and control) and (2) playback order (three levels: first, second, or third). We included all first-order interaction terms and incorporated male identity as a random effect. For model selection, in all above analyses we adopted an information theoretic approach[39]. We ranked all possible models mentioned above according to their value of Akaike's information criterion corrected for small sample size (AICc), and obtained an averaged model by selecting the most supported ones ($\Delta\text{AICc} \le 6$) after the exclusion of uninformative parameters using the MuMIn package in R[40]. Models were not over-dispersed ($\text{GLMM}_{\text{Experiment I,PC1}}$: $P = 0.936$; $\text{GLMM}_{\text{Experiment I,PC2}}$: $P = 0.96$; $\text{GLMM}_{\text{Experiment II,PC1}}$: $P = 0.92$.; $\text{GLMM}_{\text{Experiment II,PC2}}$: $P = 1.0$; $\text{GLMM}_{\text{Experiment III,PC1}}$: $P = 0.856$; $\text{GLMM}_{\text{Experiment III,PC2}}$: $P = 0.88$), no outliers were detected (all GLMMs: $P = 1$, except $\text{GLMM}_{\text{Experiment II,PC1}}$: $P = 0.285$), and visual inspection of the Q-Q plots confirmed the normality of the residuals with a single exception at the marginal significance level (Kolmogorov–Smirnov test, $\text{GLMM}_{\text{Experiment I,PC1}}$: $P = 0.046$; $\text{GLMM}_{\text{Experiment I,PC2}}$: $P = 0.671$; $\text{GLMM}_{\text{Experiment II,PC1}}$: $P = 0.336$; $\text{GLMM}_{\text{Experiment II,PC2}}$: $P = 0.964$; $\text{GLMM}_{\text{Experiment III,PC1}}$: $P = 0.705$; $\text{GLMM}_{\text{Experiment III,PC2}}$: $P = 0.570$). We applied post-hoc contrasts to differentiate between the different levels of treatments. All P values reported are two-tailed.

Results

The two studied species exhibited a similar approaching response (PC1) to playback of their own species in both sympatry and allopatry (all Experiments I–III; Tables 4, 5, 6). The tested birds flew close to the speaker during playback, and the closer they approached, the longer they stayed close (Figs. 2, 3, 4). In the site where the two

Model	Estimate	SE	z value	Pr(>|t|)
PC1—approaching response				
(Intercept)	1.05	0.37	2.79	0.005
Treatment	− 0.56	0.17	3.33	**<0.001**
Order	0.07	0.26	0.27	0.784
Order:treatment	0.21	0.17	1.21	0.227
PC2—vocal response				
(Intercept)	− 0.81	0.28	2.86	0.004
Treatment	0.42	0.09	4.62	**<0.0001**
Order	− 0.11	0.09	1.24	0.214

Table 4. Experiment I: factors and interaction terms from the generalised linear mixed models used to analyse the approaching (PC1) and vocal response (PC2) of the blue-headed wood-doves living in sympatry with congener (tambourine dove) to playbacks simulating intrusion of a stranger singing conspecific, congener, and control songs. Significant values are in bold.

Model	Estimate	SE	z value	Pr(>|t|)
PC1—approaching response				
(Intercept)	1.16	0.53	2.13	0.033
Treatment	− 0.62	0.22	2.74	**0.006**
Order	0.35	0.40	0.85	0.393
Order:treatment	− 0.32	0.20	1.52	0.128
PC2—vocal response				
(Intercept)	− 0.001	0.32	0.003	0.997
Treatment	0.17	0.16	1.03	0.302
Order	− 0.17	0.16	1.00	0.317

Table 5. Experiment II: factors and interaction terms from the generalised linear mixed models used to analyse approaching (PC1) and vocal response (PC2) of the tambourine dove living in sympatry with congener (blue-headed wood-doves) to playbacks simulating intrusion of a stranger singing conspecific, congener and control songs. Significant values are in bold.

Model	Estimate	SE	z value	Pr(>|t|)
PC1—approaching response				
(Intercept)	1.13	0.37	2.92	0.003
Treatment	-0.54	0.16	3.32	**< 0.001**
Order	-0.15	0.16	0.88	0.377
PC2—vocal response				
(Intercept)	-0.05	0.26	0.20	0.841
Treatment	0.05	0.18	0.26	0.793
Order	0.15	0.18	0.79	0.430

Table 6. Experiment III: factors and interaction terms from the generalised linear mixed models used to analyse approaching (PC1) and vocal response (PC2) of the tambourine dove living in allopatry without congener (blue-headed wood-doves) to playbacks simulating intrusion of a stranger singing conspecific, congener and control songs. Significant values are in bold.

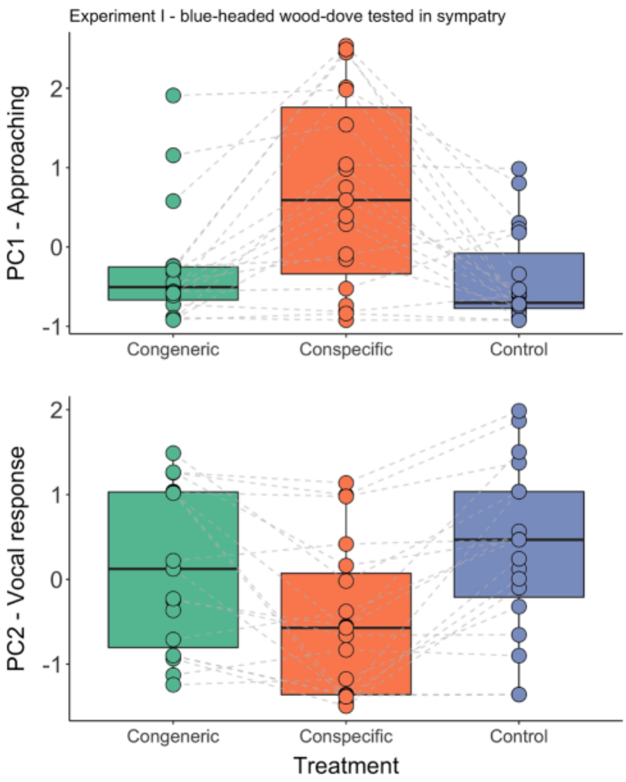

Figure 2. Experiment I: blue-headed wood-dove males responses to playback of conspecific, congeneric and control songs in sympatry (Ghana), measured with compound measures of response: PC1-approaching and PC2-vocal response.

doves are sympatric, the response pattern to the conspecific, congeneric, and control playback was very similar in both species: males approached only when conspecific songs were played, and not those of congeners or the control species (all post-hoc comparisons are presented in Table S1). In the allopatric site (no *brehmeri*), instead, *tympanistria* males demonstrated similar approach responses, and we did not find statistically significant differences in responses to playback of songs of their own species and those of the absent congener (see post-hoc tests in Table S1; Fig. 4). Furthermore, the responses to both conspecific song ($P = 0.0002$) and congeneric song ($P = 0.0005$) were significantly different from the response to the control song (details in Table S1).

The vocal responses (PC2) were less consistent among species and experiments. Males of *brehmeri* approaching the playback of their own species decreased their song rate during playback (Fig. 2, Table 4), and often continued searching for an intruder after the playback finished instead of resuming singing. Differences in vocal response to the playback of their own species compared to congeners and controls were highly significant (see Table S1). In the case of *tympanistria*, males' song behaviours were quite variable, and we found no significant differences between treatments (Tables 5, 6, S1). We found that the order of treatments had no significant

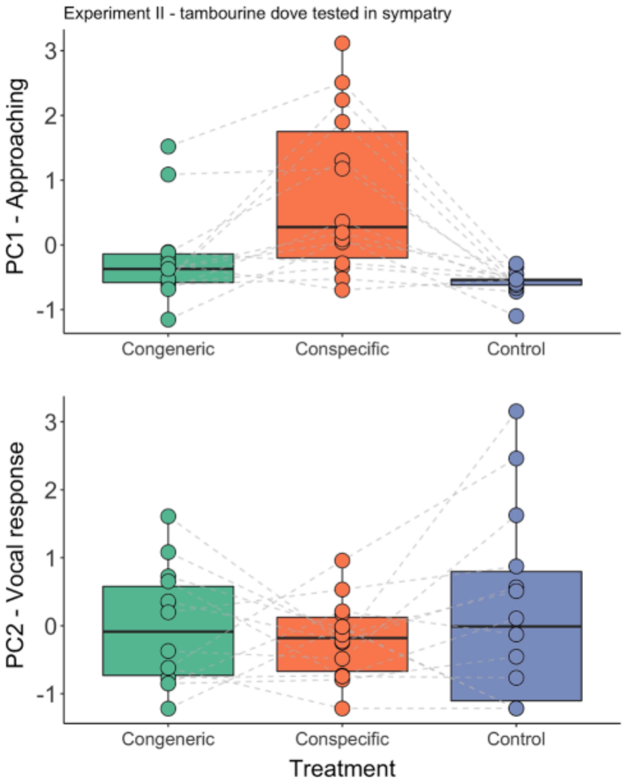

Figure 3. Experiment II: tambourine dove males responses to playback of conspecific, congeneric and control songs in sympatry (Ghana), measured with compound measures of response: PC1-approaching and PC2-vocal response.

influence on the response of tested birds; however, leaving the order out of certain models improved the results (Tables 4, 5, 6).

Discussion

In this study, we experimentally tested the effect of songs of conspecific, congeneric, and neutral control individuals on the territorial response of two closely related species of African wood doves living in sympatry and allopatry. We found that males of both species always responded strongly to the conspecific stimuli, while the single species tested in allopatry (*tympanistria*) also approached the speaker when the unfamiliar song of the absent congener was played. Neither species responded to the control song, providing a useful contrast to the responses to perceived incursions by conspecific and congeneric rivals.

Interspecific territoriality

The differences we observed between the responses to playback of conspecific song compared to congeneric song and to control song in sympatry and allopatry shed new light on the mechanisms underlying song recognition for members of the same species in addition to competition and potential interspecific territoriality in non-learning birds, represented here by African wood doves. Our results do not support the resource competition hypothesis, which posits that resources are partitioned, and defended, by dominant individuals of competing species[20]. Support for this hypothesis would be found in a strong response (with approaching as a proxy of aggression) by both species to both conspecific and congeneric song when they are found in sympatry. Instead, we found that, in sympatry, the studied *Turtur* doves only approached signals of their own species, while the response to congeneric song was similarly weak (or none) as to control song.

On the other hand, the lack of a strong response from larger *brehmeri* males to the songs of their smaller congener, together with the fact that the territories of the two species do not overlap within the sympatric range, could provide partial support for the asymmetric competition hypothesis, assuming that the larger species is dominant in interspecific territoriality[18,20,24]. As we have no information about possible hybridisation between species[34] and differences in size and colouration between *brehmeri* and *tympanistria* are substantial[28,29], we suspect that the reproductive interference hypothesis is unlikely to be relevant for this pair of species[20]. However, the apparent approaching response of *tympanistria* to the song of the absent *brehmeri* in allopatry does provide support for the misdirected aggression hypothesis[15,20].

It is not unusual to find asymmetry between the response to members of one's own species and the response to related species, or even different dialects of the same species. For example, Hamao[41] found that sympatric, related species differ in their responses to conspecific song dialects, using as models the Japanese tit (*Parus*

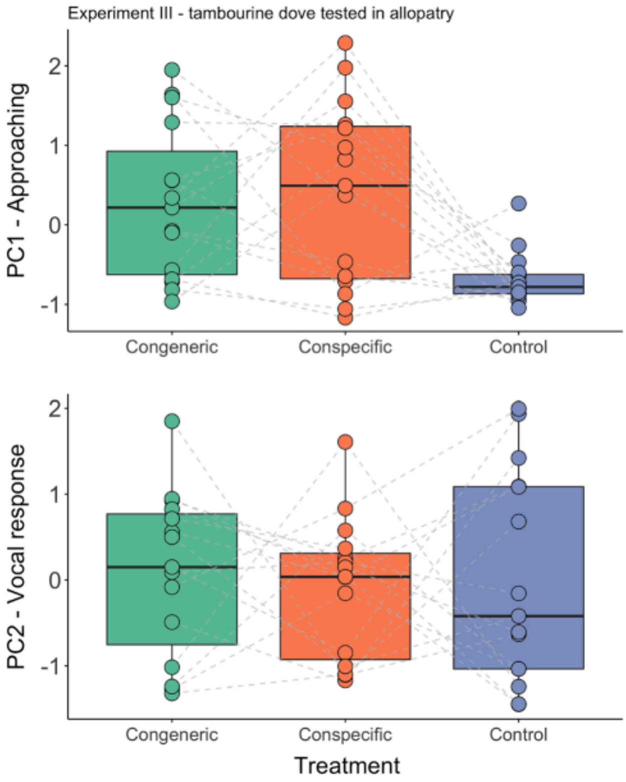

Figure 4. Experiment III: tambourine dove males responses to playback of conspecific, congeneric and control songs in allopatry (Uganda), measured with compound measures of response: PC1-approaching and PC2-vocal response.

minor) and the varied tit (*Poecile varius*). In a later study, he also found that the responses of varied tits to heterospecific songs were much weaker in sympatric populations than in allopatric ones[42]. The difference between Hamao's studies[41,42] and the present work is that the species he tested were not congeners but confamilial (*Parus* vs *Poecile)*, and that, unlike those two species, wood doves do not learn their songs[2]. The exact degree of phylogenetic distance between interacting species is likely not very important. As revealed by Drury et al. (2020), in the context of interspecific territoriality, song as a territorial signal acts at a deeper (even between-family) evolutionary timescale. Moreover, interspecific territoriality is known to occur even between unrelated species if they are forced by local conditions to compete for resources, as was observed for the chaffinch (*Fringilla coelebs*) and great tit (*Parus major*) on islands near Scotland[43]. However, interspecific interactions between non-learning birds are less known and require deeper consideration.

Is song learning important for territoriality?

Song production in doves does not involve cultural transmission[44,45]. However, it is worth remembering that there are different forms of learning in acoustic communication is worth remembering. Janik and Slater[46] distinguished between production learning and two forms of contextual learning, where individuals may produce an existing signal in a new context (usage learning) or a receiver extracts a novel meaning from a signal (comprehension learning). In both cases of contextual learning, the experience based on interactions with other individuals is crucial. So it is worth considering how potential competitors might perceive the presence or absence of a species with similar requirements and signals. Although the differences in song structure between *Turtur* spp. are small, they are consistent and allow for acoustic discrimination between species. In the case of *brehmeri* and *tympanistria*, their songs overlap in duration, number of notes, and frequency, and both species' songs can be divided into two distinct parts (own unpublished data). However, they clearly differ in the pattern of within-song note production over time (Fig. 1). In *brehmeri* songs, pauses between notes decrease constantly with time, while in *tympanistria*, pauses in the initial part of songs are variable in length. Moreover, *tympanistria* individuals differ greatly from one another in these between-note intervals, which allows for individual recognition[35]. We do not yet understand the mechanisms of species recognition in doves, particularly whether the response to conspecific songs is inherited or learned. However, pigeons are known to pay attention to the intervals between pulses, and it is likely that the above-mentioned differences in the time pattern of notes are important for species recognition[47,48]. For example, the perch-coo vocalisations of *Streptopelia* doves are long-range signals that are used for species recognition based solely on acoustic cues, and their temporal parameters were found to be the most salient features for recognition[49–51]. The song of the *Turtur* doves we studied here seems to be the functional equivalent of the perch-coo of *Streptopelia* doves, i.e. a signal that is produced to reach a receiver in the distance, who at the moment of calling is usually out of sight[52].

Doves are known to use the same brain areas for recognition of species-specific vocalisations as songbirds[53,54]. Experiments on *Streptopelia* doves indicated that they are capable of learning discrimination tasks in an operant set-up based on conspecific and heterospecific vocalisations[49,55]. Here, the contrast we observed between *tympanistria*'s lack of response to congeneric songs in sympatry and the strong response in allopatry suggests that *tympanistria* living without related species nearby cannot differentiate between the song of their own species and the song of congeners. In the allopatric site, we observed a clear movement response to *brehmeri* playback, comparable to the response to conspecifics. Hence, our results suggest that individuals with no previous experience of interacting with the congeneric species categorised songs of similar general structure as meriting a response. At least at first, when they heard the song of the congener, they did not appear to perceive it as different from their own. Consistently, this also means that the lack of *tympanistria* response to *brehmeri* song in sympatry is a form of a learned 'not responding' by the meaning of contextual learning sensu Janik and Slater[46].

Similar experiments on non-learning bird species are scarce. However, one interesting example was reported for flycatchers belonging to genus *Empidonax*. These members of the Tyrannidae family (and Suboscines) are known to develop species-specific songs without learning[56,57]. Initial studies on alder (*Empidonax alnorum*) and willow (*E. trailii*) flycatchers revealed little response to heterospecific song playback in sympatry[58]. However, when both species were tested in areas where they shared habitats (i.e., overlap in micro-scale), they were found to respond aggressively to heterospecific songs[59].

Even more remarkable was the recent finding on sympatric rallids, which are also non-learners (phylogenetically even more distant from any song-learning bird taxa than Tyrannids). Jedlikowski et al. (2022) showed that the water rail, *Rallus aquaticus*, and the little crake, *Zapornia parva*, are able to distinguish not only each other but also specific individuals of the other species. This work provided an example of a dear-enemy effect in different species. Based on these studies, it seems that being a non-learner in the context of the acquisition of vocalisation (socially learned vs inherited) does not necessarily limit a bird's abilities to diversify its responses toward different individuals of the same and even other, potentially competing, species.

The response to heterogenic song was also tested experimentally in other African dove taxa, although in a different context than in our study: hybridisation. In Uganda, de Kort et al.[52,61] studied two closely related doves (vinaceous dove, *Streptopelia vinacea*, and ring-necked dove, *S. capicola*) as well as hybrids between the two. The authors focused on perch-coo and bow-coo signals, which both play a role in inter- and intra-sexual signalling but are produced in behaviourally different contexts (long vs short distance communication). They found that allopatric populations showed a stronger response to conspecific than to heterospecific perch-coos, but equal responses to bow-coos of either species. Instead, hybrids exhibited no clear pattern between their own coo structure and that of the species to which they responded most strongly, indicating a lack of behavioural coupling. Interestingly, in allopatry, ring-necked doves responded more strongly to perch-coos of vinaceous doves than vice versa, which the authors explained based on their ecological history. Namely, the ring-necked dove had expanded into the vinaceous dove's range, thus creating a context in which rapid learning of a novel (heterospecific) competitor's song might be favoured[52].

Overall, then, research on other non-learning bird species has revealed flexibility in their response patterns to conspecific and congeneric (or even more evolutionary distant) individuals depending on the ecological and evolutionary context.

An ecological perspective on interactions between wood doves

In the sympatric area, where *brehmeri* and *tympanistria* co-occur and their territories are often adjacent, we observed no approaching response to both congeners' songs and control songs in our experiments. From several observation posts, males of both species could be heard simultaneously, but the distance between song posts was typically more than 50–100 m, and their territories seemed not to overlap. In several patches, only one of the two species was present. These observations have two potential explanations: (i) both species can discriminate between the songs of conspecifics and those of congeners, or (ii) the weaker competitor can discriminate, and it uses this information to make decisions regarding settlement in sympatry. Our field data suggest that in Kakum NP, *brehmeri* males choose territories deeper in the forest, containing the highest trees close to streams, while *tympanistria* were often found close to forest edges or partly open areas, e.g., in tree fall gaps (or secondary forest). In Kibale NP, instead, *tympanistria* were most often found in sites like those preferred by *brehmeri* in Kakum NP. This suggests that, in sympatry, smaller *tympanistria* males may avoid settlement close to (or within the territory of) *brehmeri*, perhaps as the result of initial competitive interactions with their congeners. Such a mechanism would support the asymmetric competition hypothesis, with the caveat that the birds do not have to compete all the time. Data on the life history of wood doves are scarce but based on re-captures and recording the same birds in the same territories year after year, it appears that their territories are stable for a long time, maybe even their whole lives. Like many birds in the tropics, they are likely to be long-lived if they succeed in reaching adulthood[62]. This may suggest that the response pattern to congeners (and members of their own species) is settled when young males try to establish their territories for the first time. Based on this, and on the observations we made in our experiments, we hypothesise that when the two species are found in sympatry, young *tympanistria* likely interact with *brehmeri* males but are chased away by the stronger rival, and thus avoid such confrontations later in life. The ability to discriminate between conspecific and congeneric individuals is thus acquired through life experience, enabling the bird to save time and energy later in life. This is a hypothetical but probable scenario for the coexistence of these two species in sympatry, which also explains the pattern of responses observed in allopatry.

Evolutionary perspective (functionality of response to congener's song in allopatry)

Our results suggest that tambourine doves may have some inherited template memory, but that response control is likely to develop while listening and interacting with potential rivals and/or mates. Therefore, we can assume that in sympatry, *tympanistria* must have learned not to respond to a song of a stronger (congeneric) rival (comprehension learning)[46]. This ability to learn responses to songs similar to their own opens the door for between-species interactions and territoriality in case of changes in species range. From this point of view, we should reconsider whether or not responding to a sister species in the allopatric zone can truly be considered maladaptive, as argued by the misdirected aggression hypothesis. In Kibale NP, where the congener is not naturally found, this response could indeed be viewed as maladaptive. Still, from a long-term and broad-scale perspective, this pattern may represent a plastic ability to find the best response in a changing environment that may or may not contain individuals of both species. Therefore, the misdirected aggression hypothesis may not be, by definition, maladaptive. Species ranges can change as a result of many factors, including in some dove species[63,64], and the ability to adapt (i.e., to respond functionally) to a new competitor may be crucial on the evolutionary time-scale. In this context, our experimental results are consistent with a recent comparative study that revealed, through a large-scale phylogenetic analysis, that interspecific territoriality is widespread in birds and is strongly associated with hybridisation and breeding habitat overlap[18].

The pair of *Turtur* species studied here seems to be a good model for investigating responses to heterospecific song under different forms of allopatry. *T. tympanistria* has a larger species range and less-specific habitat preferences than *brehmeri*; it can thus be found at a distance of hundreds of kilometres to the closest *brehmeri* population, as well within basically the same area but at different elevations, such as on Mount Cameroon[28,29,65]. It would be extremely valuable to understand how *tympanistria* from different allopatric populations respond to the song of congeners. For example, a study on sibling species of African sunbirds revealed that responses to heterospecific song could be strongly different among allopatric populations[66]. Conversely, another tropical species, the white-eared ground-sparrow (*Melozone leucotis*) used information encoded in vocalisations to discriminate competitor from noncompetitor species even in the absence of previous experience (understood as living in sympatry or allopatry with Prevost's ground-sparrow, *Melozone biarcuatum*)[67]. From these previous studies and the current work, it appears that the responses of birds to heterospecific song are diverse and cannot be generalised to a single common pattern.

Conclusions

Two species of *Turtur* wood doves living in sympatry, where they do not exhibit interspecific territoriality (at least as adults with established territories), responded strongly to songs of their own species but did not approach the song of congeners and control songs. In allopatry, instead, *tympanistria* males 'misdirected' their response to the congeneric dove song, even though they had not previously had any earlier contact with the species. Their approach response to the playback of *bremeri* song was similar to that of their own species, while they did not approach control songs. We suggest that this is not necessarily a totally maladaptive behaviour as, from an evolutionary perspective, such an ability could be useful for resolving competing interests with congeners. It is more adaptive to be plastic in the response to a potential competitor than to respond or not respond in a fixed way.

Data availability

The datasets analysed during the current study are available from the corresponding author on reasonable request (Tomasz S. Osiejuk, email: osiejuk@amu.edu.pl).

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Author contributions

T.S.O. conceived this project; M.N., P.S., M.B. and T.S.O. collected data and performed experiments. M.N. and T.S.O. performed the bioacoustics and statistical analyses; P.S. was responsible for collecting all permits and contact with local authorities; M.N. and T.S.O. drafted the manuscript and all authors contributed to the editing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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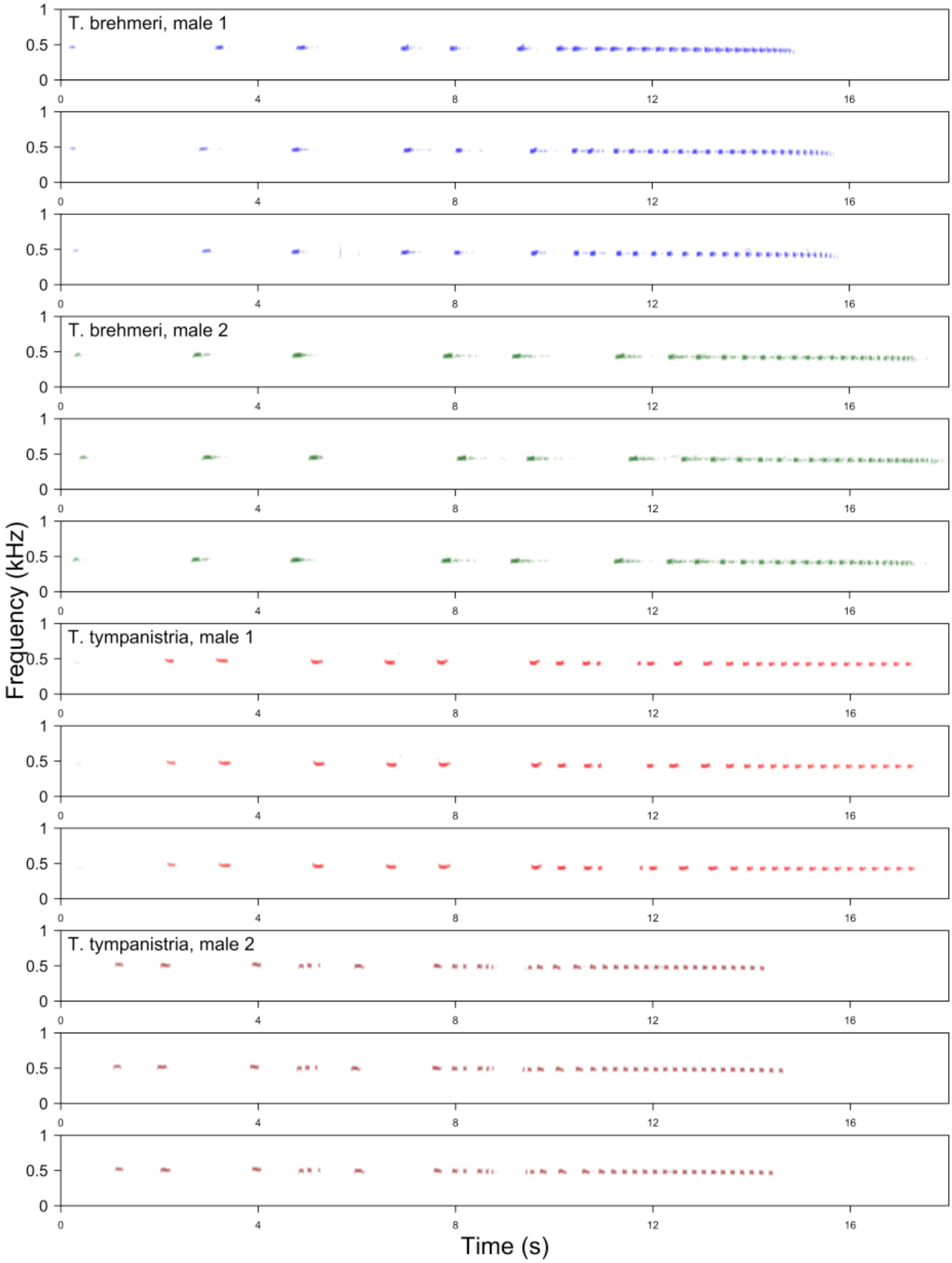
Table S1. The contrast linear comparisons of PC1 - Approaching response and PC 2 - Vocal response in Experiments I to III. Post-hoc tests were conducted to compare pairs of treatments within each experiment, i.e., Conspecific vs Congeneric, Conspecific vs Control and Congeneric vs Control.

Experiments and post-hoc comparisons for response variables (PC1, PC2) tested	Estimate	SE	χ^2	$P > \chi^2$
Experiment I				
Model PC1 - Approaching response				
Conspecific vs Congeneric	1.03	0.21	23.76	**< 0.0001**
Conspecific vs Control	1.12	0.21	28.79	**< 0.0001**
Congeneric vs Control	0.09	0.21	0.20	0.651
Model PC2 - Vocal response				
Conspecific vs Congeneric	-0.65	0.16	16.59	**< 0.0001**
Conspecific vs Control	-0.83	0.16	27.17	**< 0.0001**
Congeneric vs Control	-0.18	0.16	1.22	0.268
Experiment II				
Model PC1 - Approaching response				
Conspecific vs Congeneric	1.01	0.27	13.86	**0.0002**
Conspecific vs Control	1.36	0.25	30.12	**< 0.0001**
Congeneric vs Control	0.34	0.27	1.64	0.201
Model PC2 - Vocal response				
Conspecific vs Congeneric	-0.01	0.31	0.00	0.982
Conspecific vs Control	-0.19	0.28	0.48	0.488
Congeneric vs Control	-0.19	0.31	0.38	0.537
Experiment III				
Model PC1 - Approaching response				
Conspecific vs Congeneric	0.06	0.27	0.04	0.833
Conspecific vs Control	1.01	0.28	13.50	**0.0002**
Congeneric vs Control	0.96	0.27	12.17	**0.0005**
Model PC2 - Vocal response				
Conspecific vs Congeneric	-0.12	0.35	0.11	0.736
Conspecific vs Control	-0.06	0.35	0.03	0.859
Congeneric vs Control	0.05	0.35	0.03	0.873

Figure 1S. Spectrograms illustrating individual differences between males of the blue-headed wood-dove (*Turtur brehmeri*) and the tambourine dove (*Turtur tympanistria*). Different colours indicate the songs of the same individuals.



CHAPTER 3

Response of the emerald-spotted wood-dove to the song of conspecific males and sympatric congeners

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Ethology, manuscript in revision

Response of the emerald-spotted wood-dove to the song of conspecific males and sympatric congeners

Short title: Wood-dove response to conspecific and sympatric congeners song

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Abstract

Bird song contains crucial information that enables recognition of conspecific individuals from a distance, which facilitates subsequent complex behaviours related to mate attraction and rival deterrence. Loud broadcast signals of non-learning bird species are usually treated as less complex than songs of Oscines, but several studies have revealed that song learning is not necessary for the evolution of complex signalling. Here, we focused on a species of African wood-dove which produces superficially simple songs consisting of short notes organised into two parts of different amplitude. Our model was a population of emerald-spotted wood-doves (*Turtur chalcospilos*) that live in the same area as the physically and vocally similar blue-spotted wood-dove (*Turtur afer*). We tested the responses of male emerald-spotted wood-doves to different playbacks simulating territorial intrusion. We used songs of the focal species and of the congener, two types of artificially mixed songs with different parts belonging to the focal or congeneric species, and a control song. We aimed to assess (i) whether the focal species responds only to its own species' song or exhibits interspecific territoriality, and (ii) which part of the song is responsible for coding species identity. We found that male emerald-spotted wood-doves responded strongly to playback, but almost exclusively only approached playback of their own species' song. Additionally, only conspecific song caused a decrease in song rate during playback and an increase in song output after playback. Our results suggest that emerald-spotted wood-doves are able to discriminate their own songs from those of congeners and do not exhibit interspecies territorialism. We were unable to identify a single part of the song that codes species-specificity, as mixed songs of any kind did not substantially increase responsiveness to playback. We discuss these results in the context of current hypotheses regarding interspecific territoriality and the evolution of species-identity coding.

Keywords: allospecific communication, bird song, species recognition, sympatric occurrence, territorial signalling, emerald-spotted wood-dove, *Turtur chalcospilos*

Introduction

General introduction

Acoustic communication plays a key role in the survival and reproductive success of many animal species, and it has evolved in exceptionally diverse forms. These range from simple vocalisations, such as drumming sounds in fish caused by changes in the pressure of the swim bladder (Kasumyan, 2008) to complex songs in songbirds, which use a duplex sound production apparatus (syrinx) to simultaneously produce independent, acoustically distinct vocalisations (Goller, 2022). Communication between individuals helps in a wide variety of social behaviours, such as establishing social bonds and hierarchies (Fischer & Price, 2017), coordinating group activities like hunting (Drea & Carter, 2009), informing about a food source (Evans & Evans, 1999), or warning other individuals of potential danger (Ey & Fischer, 2009; Semple & Higham, 2013). In birds, the most crucial roles of vocal communication are to defend territory and attract a mate (Catchpole & Slater, 2008). These two functions often depend on each other, as a high-quality territory is often a prerequisite for attracting a mate. A male who successfully defends his territory may be evaluated more favourably than competitors by a female, increasing his likelihood of achieving reproductive success (Dalziell, Welbergen & Magrath, 2022; Wang *et al.*, 2022). Territory defence in birds is inevitably linked to acoustic communication. To be most effective, territory owners should be able to detect signals from a distance and properly assign them to the species, sex, and individual of origin. They should also be able to determine the location of the sender and decipher information about the signaller's quality, motivation, and intention (Catchpole & Slater, 2008). In this way, the receiver can ensure that the cost of defence does not exceed the value of the defended resources. Therefore, any evaluation of the mechanisms of acoustic defence must be grounded in an understanding of the relationship between a signal's structure (or the pattern of its production) and the response it provokes.

For territorial birds, the first functional level of acoustic signalling is recognising whether the signal heard was produced by a conspecific individual or not. In general, effective recognition of one's own species is crucial for decision-making. This is especially true for birds, as they often communicate over long distances (frequently with an effort to maximise the distance of signal transmission; e.g., Benedict & Warning, 2017), and many decisions, at least initially, have to be made solely based on the acoustic properties of a received signal (Catchpole & Slater, 2008). Territorialism and acoustic defence are mostly associated with competition between males belonging to the same species. The first line of territory defence often features songs or other territorial signals like calls or drumming, and experiments involving the removal and muting of males have explicitly revealed this function (Catchpole & Slater, 2008). In order for a male's song to deter a conspecific rival, the receiver must be able to distinguish such signals from the background noise. This assumption is consistent with hypotheses regarding both species recognition and acoustic niche adaptation (Hutchinson, 1959; Seddon, 2005), which posit that species living in sympatry should produce signals that minimise the risk of hybridisation and occupy unique acoustic niches to avoid interspecific competition. However, closely related species are, by definition, similar to each other

(Bradbury & Vehrencamp, 2011), and species living in similar habitats may evolve analogous signals adapted for transmission in their environment (Morton, 1975). Therefore, long-range signals in birds evolve under a set of pressures that may sometimes have opposite effects. Recent studies have revealed that when different species hybridise or target the same resources, it may result in a particular type of interspecific territorialism (Cowen, Drury & Grether, 2020; Drury, Cowen & Grether, 2020), that is, behaviours by an individual of one species intended to defend its territory against individuals of another species. Recently, this has been recognised in an increasing number of species, including those that are phylogenetically distant from each other (Losin *et al.*, 2016; Drury *et al.*, 2020), and its origins have been suggested by four hypotheses related to resource competition, asymmetric resource competition, reproduction, and misdirected aggression (reviewed in Cowen et al. al., 2020). When studying territorial signalling in closely-related, sympatric species, then, it is important to investigate if interspecies territorialism might be at work, by determining whether and how related species respond to each other's signals.

To test hypotheses related to intra- and interspecific territoriality and acoustic signalling, African wood-doves from the genus *Turtur* are a particularly useful model. These birds inhabit a range of habitats, from lowland equatorial forests to savannah and miombo, ending at the semidesert areas along ~15°N latitude. The individual species have different ranges and habitat preferences, but in many areas, two or three species co-occur. This provides a unique opportunity to study interactions between closely related species living along a gradient of habitats that affect the evolution of signals. The genus comprises five species characterised by very similar broadcast territorial vocalisations, hereafter called songs. Song phrases are relatively long (average duration 11–16 s, but may even reach 35 s), consist of many (14–54) simple low-frequency notes, and have two distinct parts (hereafter termed initial and final) that are characterised by variation at both the species and individual levels. In all wood doves, the initial part of the song is quieter. However, in birds that occupy more open habitats, initial notes have, on average, 6–12 dB lower amplitude (and can be even > 30 dB lower) than the loudest song part. This dramatically decreases the distance from which the entire song can be heard (Osiejuk et al. in preparation). For an inexperienced human listener, recognising a species by its song can be challenging. It is possible that birds experience similar confusion, as studies have revealed that, under certain circumstances, doves may respond to the song of a related species. For example, the endangered pink pigeon, *Nesooenas mayeri*, which is endemic to Mauritius, was found to respond to playback of the similarly cooing invasive Madagascan turtle dove, *N. picturata* (Wolfenden *et al.*, 2015). Likewise, tambourine doves, *Turtur tympanistria*, responded to playback of a congener's song in sites in Uganda where the latter species does not naturally occur (Niśkiewicz *et al.*, 2023).

In this study we primarily focused on the emerald-spotted wood-dove, *Turtur chalcospilos*, which is found in a variety of woodland habitats such as riparian forests, savannah, and miombo, as well as secondary bush or gardens around villages in eastern Sub-Saharan Africa (Baptista *et al.*, 2020b). It often co-occurs with the blue-spotted wood-dove, *T. afer*, which has more a west-central distribution and prefers wetter habitats (Baptista *et al.*, 2020a). In this study, we examined sites that host both species of doves and where interactions between the two species may potentially occur on a daily basis. In these locations, both species are certainly able to hear each other’s songs, and literature data indicate that they are capable of hybridising (McCarthy, 2006a), with the hybrids being fertile (Brickell, 2005).

Aims of the study

We investigated whether and how emerald-spotted wood-dove males who live in sympatry with the blue-spotted wood-dove are able to discriminate between the songs of their own species and those of the congener. As these two species are morphologically very similar, have similar habitat requirements and overlapping distribution ranges (Baptista *et al.*, 2020a, 2020b), and are known to hybridise (Brickell, 2005; McCarthy, 2006b), it is possible that they exhibit interspecific territoriality (Losin *et al.*, 2016; Drury *et al.*, 2020). If that is the case, we would expect the tested males to respond with similar intensity to both the song of their own species and that of the congener. On the other hand, closely related bird species living in sympatry may avoid competition and hybridisation by choosing different micro-habitats (syntopic allopatry), and in this case, long-distance signalling can help reduce unnecessary (physical) interactions (Cowen *et al.*, 2020). In this scenario, we would expect that the emerald spotted wood-doves recognise from a distance only rivals from their own species and respond with increased aggressiveness only to conspecific song. As mentioned above, *Turtur* doves' songs consist of two distinct parts, which may potentially carry differentiated information about species and individual identity. Therefore, we tested the birds' response not only to complete songs of their own species and the congener, but also to artificially mixed songs in which either the initial or final part of the song originated from the congener. We expected that male emerald-spotted wood-doves would exhibit a stronger response to whichever mixed song phrases they identified as conspecific songs. By comparing the different mixed songs and the responses they provoked, we aimed to determine whether the initial or final song part carries a “species identity” message, with our hypothesis being that the louder final part (in this species, on average +12 dB SPL) is more likely to contain species-specific information. The higher amplitude of this part of the signal should extend the range covered, which would benefit the sender from the perspective of both territory defence and mate attraction.

To answer these questions, we conducted playback experiments simulating territorial intrusions and measured the behavioural response of emerald-spotted wood-dove males to (i) the song of their own species, (ii) the song of the congeneric (and sympatric) blue-spotted wood-dove, (iii-iv) two artificially created song stimuli consisting of different combinations of the initial and final parts of the song of the focal and congeneric species, and finally, (v) to a control song of a sympatric but unrelated dove species from a different genus. We predicted that these two species are likely to exhibit interspecific territoriality, and thus that the emerald-spotted wood-dove would respond to the song of the congener. If it did not, we expected birds to react to one of the artificially created mixed songs, depending on which part of the song contains species-specific information.

Methods

Study site and species

The study was conducted in Lake Mburo National Park in Uganda (0°37' S, 30°58" E, altitude 1239–1607 m asl). Lake Mburo NP is the smallest national park in Uganda but contains quite a diversified mixture of habitats, with rocky ridges, forested gorges, narrow bands of riparian woodland, and grassland with acacia trees. There are both seasonal and permanent swamps. The area of Lake Mburo NP receives an average annual rainfall of 874 mm, with most precipitation occurring from March to May and from September to November. Daily temperatures fluctuate around 27°C, with night-time averages reaching around 14°C. All recordings and experiments were conducted in an area up to 7 km south and 8 km east of the Sanga Gate, in habitats transitioning from riparian woodland (along the western shore of Lake Mburo) to grassland (area between Lake Mburo and Kachira Lake).

The emerald-spotted wood-dove is widely distributed in Lake Mburo NP and is typically found in most habitat types. Based on our data, which include recordings of birds along transects, point recordings, and captures, the congeneric species—the blue-headed wood-dove—appears to be at least three times less frequent within the study area. We also noticed and captured tambourine doves in the area, but they are much less numerous and prefer more wooded parts of the park.

Preliminary fieldwork

Prior to the experiments, in June and July of 2020 and 2021, birds were observed in order to gather information about their territorial behaviour, song post locations, and natural interactions between males. Practices also included mist-netting, colour-ringing, and attaching radio tags to a fraction of males (18 individuals) to confirm that their song structure remains unchanged while singing. To verify individual vocal distinctiveness, we tracked males with radio tags and recorded their song.

Recordings of birds were taken using high-quality digital recorders: Marantz PMD661 MK II (Marantz Professional, Kanagawa, Japan), Sony PCM-D100 (Sony Corp., Tokyo, Japan), and MixPre-3 recorders (Sound Devices, Madison, Wisconsin, USA), coupled with a Sennheiser ME67 or MKH70 shotgun microphone (Sennheiser, Wedemark, Germany), at a 48-kHz sampling frequency and 24-bit resolution. The maximum amplitude of four spontaneously singing males was measured with the use of a CHY 650 digital sound level meter (CHY Firemate Co., Ningbo, China) at a distance of 6–10 m (measured with a Leica DISTO D510 laser range finder). After recalculation for a 1-m distance, we found that the average maximum amplitude was 68 dBA, with a range between 62 and 77 dBA SPL (95CI: 65–71 dBA SPL).

Sound samples and playback preparation

Based on our preliminary fieldwork, we prepared song samples for playback experiments. We selected songs with a high signal-to-noise ratio, which was assessed visually using a sound spectrogram. All recordings were filtered (typically high-pass, 0.1 kHz; low-pass 1.5 kHz) before preparing playback sound samples. The experiments used songs of emerald-spotted wood-doves, blue-spotted wood-doves, and, as a control, the locally common ring-necked dove, *Streptopelia capicola*. We did not observe any aggressive interactions between the ring-necked dove and the emerald-spotted wood-dove during preliminary observations. Each playback stimulus was created from a single song phrase in order to obtain a natural-sounding output. Playbacks were prepared from samples from different individuals from the opposite side of the study area, at a distance of 3 or more km, to avoid pseudo-replication and ensure that all tested birds responded to unknown individuals each time. During each treatment, we played all dove songs with the same natural rate of 1 song phrase every 30 seconds. The volume of each playback was set to 68 ± 2 dBA SPL at a 1-m distance from the speaker (measured with a CHY 650 digital sound level meter; CHY Firemate Co., Ningbo, China). Digital editing, construction, and analysis of the playback files were conducted with Raven Pro 1.6 (Cornell Lab of Ornithology, Ithaca, USA) and Avisoft SASLab Pro 5.2 and later versions (Raimund Specht, Berlin).

Playback experiment design and procedures

The experiments were conducted in June 2022. Field work started on the 13th of June 2022, and playback experiments were performed between the 18th and 25th of June 2022 between 6:47 and 10:30 local time (sunrise at ~ 5:57). We intentionally avoided testing birds during the dawn chorus as well as during the hottest part of the day, which usually begins around 11. Altogether, we tested 73 different males of the emerald spotted wood-dove. Each male was tested once, using one of the five types of playback: (1) TC – conspecific song (non-neighbour from the local population), (2) TC-TA - mixed song consisting of the initial part of the conspecific song and final part of the congeneric song; (3) TA-TC - mixed song consisting of the initial part of the congeneric song and final part of the conspecific song; (4) TA - song of the congeneric species (i.e., the blue-spotted wood-dove from the local population); and (5) SC - control song of another sympatric dove species, the ring-necked dove (recorded in the local population) (see Figure 1). Hereafter, these abbreviations are consistently used to denote the different playback treatments.

Experiments were only conducted when the focal male was present and singing before playback. The speaker was placed 25–35 m from the focal male in a way that allowed him to approach the speaker and sit close to it without approaching the observers. The songs were played back as a WAV file with a Tascam DR-05 (TEAC Europe GmbH, Wiesebaden, Germany) or Sony PCM-D100 (Sony Corp., Japan) and from a JBL Charge 4 speaker (Harman International Industries, Stamford, Connecticut, USA) that was placed on branches ca. 2 m above the ground.

When observing a singing male, one person started to record the bird from a distance (for about a minute) while the second observer placed the speaker on a tree and initiated the playback experiment. There was a short delay before playback began to let the observer move away quietly and take a good position for the observation of behaviour. The playback phase consisted of 10 songs presented for 5 minutes. The focal bird was then observed further for a 5-minute post-playback period. All vocal responses of the tested birds were recorded by one of the two observers positioned ca. 20 m from the speaker using a Sound Devices Mix-Pre3 recorder with one of the Sennheiser shotgun microphones. The person recording songs had an additional lavalier microphone connected to the second channel of the recorder for dictating descriptions of the bird's behavioural responses and other observations. The second observer was on the opposite side of the speaker in a location convenient for observing the experimental scene. Recordings of vocalisations and physical behaviour were later synchronised based on a 'beep' sound that was included in playback at the end of the post-playback period. This sound was well above the doves' song frequency and was similar to the contact calls of small birds. All distances reported (as the closest distances to a speaker) were measured with a Leica DISTO D510 laser range finder.

To describe the behavioural responses of tested males, we measured the time spent within 10 m of the speaker (s), latency to approach the speaker (s), the closest approach to the speaker (m), and the number of flights during and after playback. If the focal male only moved away from the initial position towards the speaker, the larger distance was recorded as the closest approach. The numbers of songs sung during and after playback were extracted from the recordings of focal males.

Ethical Note

To our knowledge, the individuals tested in the experiment reflected the population in a representative way with no potential biases resulting from social background, self-selection, habituation, or other factors, as indicated in the STRANGE framework (Webster and Rutz 2020). Our experimental procedure adhered to ASAB/ABS Guidelines for the care and use of animals (The Ethics Committee (ASAB) and the Animal Care Committee (ABS), 2019) and was approved by all responsible local bodies mentioned below as well as by the Polish Laboratory Animal Science Association (certificate no. 1952/2015 to TSO), conforming to Directive 2010/63/EU. Our experimental procedures were approved in Uganda by Makerere University, College of Health Sciences; Makerere University Biological Field Station; Uganda Wildlife Authority, permit no. COD/96/05; and the Uganda National Council for Science and Technology, permit no. NS256ES.

Statistical Analysis

The original response variables we measured during experiments were partly correlated with each other, but the multicollinearity was moderate (variance inflation ratio

(VIF) between 1.20 and 3.42). Therefore, to analyse the general strength of a response to playback, we used a principal component analysis (PCA) with varimax rotation and Kaiser normalisation (IBM SPSS Statistics 28.0.1.0). The dataset was evaluated and found to be appropriate for PCA (Kaiser-Meier-Olkin = 0.74, Bartlett test of sphericity = 215.826, $P < 0.001$). The first component explained 45.27% of the variance and had heavier loadings from all three variables related to the approach to the speaker (Table 1). We refer to this principal component as PC1 - Approaching. The second principal component explained 24.53% of the variance and had heavier loadings from both variables related to singing (Table 1). We refer to this as PC2 - Vocal response component.

Higher values of PC1 indicated more flights, a closer approach to the speaker, and staying longer in its vicinity, hence, a stronger response. Higher values of PC2 indicated that the responding bird was singing more both during and after playback. In the case of PC2, a stronger response was not linked with higher values, as males approaching the speaker sometimes stopped singing. Hence, the interpretation of PC2 - Vocal response depended on the other activities of the responding bird. Because of this, we also compared the numbers of song phrases sung during and after playback (see below). We found that the compound response variables deviated significantly from normality (both $P < 0.01$), and in the case of PC1, the variable was also strongly positively skewed. Therefore, we used the non-parametric Kruskal-Wallis test to determine if the responses measured with PC1 and PC2 differed significantly between treatments. When significant differences were detected, we compared pairs of treatments using the NPTEST command of SPPS and adjusted significance values with Bonferroni corrections.

To check for differences between the number of song phrases sung during and after playback, we used a generalised mixed model with a log-link function and a Poisson error structure implemented in the glmmTMB package v. 1.1.7 (Brooks *et al.*, 2017). We used the number of songs produced as our response variable and included treatment (five levels: TC, TC-TA, TA-TC, TA, and SC), phase of the experiment (two levels: playback and post-playback), and the interaction term between treatment and phase as the main factors, with the identity of the male incorporated as a random effect. Model assumptions were checked using the DHARMa package v. 0.4.6 (Hartig, 2020). All *P* values reported are two-tailed.

Results

We conducted playback experiments with 73 different males of the emerald-spotted wood-dove; of these, we tested 14 with TC playback, 16 with TC-TA playback, 17 with TA-TC playback, 14 with TA playback, and 12 with control playback (SC).

We found that the most characteristic sign of a strong response was immediate flying towards the speaker. Males responding to the full song of their own species approached the speaker in all cases except one (Table 2). In contrast, when we played back the control song of a ring-necked dove, they rarely approached the speaker; moreover, these approaches were not close, but instead appeared to be accidental (Table 3). In the treatments with mixed songs and the song of the congeneric species, the percentage of males that approached the speaker was higher than for the control, but less than for the TC treatment (Table 2). These differences in behaviour (approaching the speaker or not) were statistically significant among groups (Kruskal-Wallis test, χ^2 = 12.968, df = 4, P = 0.014).

We evaluated these behaviours in depth using a principal component analysis, and found that PC1 - Approaching scores differed significantly depending on the playback type (Kruskal-Wallis test, χ^2 = 25.576, df = 4, $P < 0.001$). The highest values were obtained for the conspecific song treatment, meaning that those males approached the speaker faster, performed more flights, and remained within 10 m of the loudspeaker for a longer time than males in other treatments (Figure 2a). The post-hoc tests comparing pairwise differences with Bonferroni correction indicated that the TC treatment, and only this treatment, was significantly different from other treatments (vs. TC-TA, P = 0.019; vs. TA-TC, P = 0.001; vs. TA, P = 0.015; and vs. SC, $P < 0.001$). All other paired comparisons were statistically insignificant (all $P \geq 0.679$)

There were no statistically significant differences in scores for PC2 - Vocal response among the treatments (Kruskal-Wallis test, χ^2 = 5.049, df = 4, P = 0.288). However, birds responding to song of their own species (treatment TC) or any song that contained at least a part of the species-specific song (treatments TC-TA and TA-TC) tended to sing more during and after playback (higher values of PC2), while those responding to congeneric or control song sung less (lower PC2; Figure 2b). To shed more light on this, we performed a complementary analysis that directly compared the number of songs sung during and after playback (see Figure 3). The mixed model (Songs ~ Treatment + Phase + Treatment × Phase + (1|Individual)) revealed no significant effect of treatment ($\beta \pm$ SE: -0.33 ± 0.53, z = -0.62, P = 0.536) or phase of the experiment ($\beta \pm$ SE: -1.00 ± 1.4, z = -0.71, P = 0.479) on the number of songs sung by a focal male. However, we did find a significant effect of the interaction between the treatment and experimental phase: males who were responding to their own species' songs (treatment TC) significantly increased their song production rate after playback compared to during the playback phase ($\beta \pm$ SE: 3.89 ± 1.92, z = 2.02, P = 0.043; Figure 3).

Overall, we observed a generally clear pattern of decreasing response intensity among the treatments, with TC > TC-TA > TA-TC > SC. However, there were some outliers in almost all treatment groups. In the TC treatment, there was an obvious approach response from all males except one, who may have been frightened by one of our rangers. In both of the mixed song treatments, the focal males stayed on average much further away than in the TC treatment, but sometimes approached and remained closer to the speaker. Finally, we observed a single male who responded to TA playback with an exceptional number of flights, and, as mentioned above, recorded what were likely accidental flights in the direction of the speaker following playback of ring-necked dove song.

Discussion

Summary of results

Altogether, we observed a clear response from male emerald-spotted wood-doves only in the TC treatment: these males obviously approached the speaker, reduced their song rate while flying towards the simulated rivals, and subsequently increased their song rate once the playback concluded. In both mixed treatments, males maintained a consistent song rate during and after playback, without approaching the speaker in most cases. Finally, males responding to both the congeneric song (TA) and control playback (SC) usually did not approach the speaker and reduced their song rate as the experiment progressed. It appears that in these two latter treatments (TA, SC), this decrease was associated with a diminished internal motivation to sing, regardless of the playback. During the experimental observations, a stark contrast was evident between the TC treatment, in which birds flew to the speaker after almost every playback, and the TA and SC groups, which were essentially motionless.

Emerald-spotted wood-doves recognise the song of their own species and do not respond to the song of a congener

We found that male emerald-spotted wood-doves living in sympatry with blue-spotted wood-doves unequivocally demonstrated a strong response only to playback of their own species' songs. When they were presented with the congener's song, their behaviour was statistically indistinguishable from observations following the playback of a control song, i.e. from a ring-necked dove. We also found that artificially mixed songs in which the final part was taken from a congeneric individual did not contain enough clearly species-specific signals to evoke a complete response. However, in a few cases, we did observe emerald-spotted wood-dove males approaching this kind of playback. The results we obtained indicate that, within the study area, there is no interspecific competition between emerald-spotted and blue-spotted wood-doves in response to singing, at least in the sense that the emerald-spotted wood-dove males do not appear to regularly chase away vocalising blue-spotted wood-doves.

Our results also demonstrated quite clearly that male emerald-spotted wood-doves do not interact vocally with their sympatric congener. There are many potential reasons for this. Despite the many similarities between these two species, they may not compete for precisely the same resources, or the intensity of competition could be reduced because their peak breeding periods do not overlap. Data on the phenology of these species suggest that April–June is the preferred nesting period for the emerald-spotted wood-dove (Baptista *et al.*, 2020b) in East Africa, while the blue-spotted wood-dove prefers October–March (Baptista *et al.*, 2020a). However, nests of both species have been found in almost all months of the year (Baptista *et al.*, 2020a, 2020b). Within the study area, the emerald-spotted wood-dove is certainly more abundant than its congener, and we found evidence of breeding during our field work (nests with eggs). The blue-spotted wood-doves were less commonly observed singing, but this was probably more a reflection of their lower densities rather than their true activity patterns, as the proportions of birds that were heard and mist-netted were similar in both species. A comparable situation, in which congeneric *Turtur* species were found to not respond to each other's songs, was observed for the blue-headed wood-dove, *Turtur brehmeri*, and the tambourine doves inhabiting lowland forest in Ghana (Niśkiewicz *et al.*, 2023). In this case, males of these two species were clearly found within the active range of each other's songs. However, they seemed to prefer slightly different microhabitats, were not observed to interact with each other, and did not respond to playback of congeneric song. Instead, the opposite situation was observed in an allopatric site in Kibale NP in Uganda, where only the tambourine dove is present. In this population, male tambourine doves did approach playback of the song of the unknown (in this location) blue-headed wood-dove (Niśkiewicz *et al.*, 2023). This study suggests that, regardless of being vocal non-learners (in the sense of learning vocal production), doves do learn to respond to signals, and their responsiveness may depend on their experience and the presence of potential rivals. In the abovementioned case from Uganda, tambourine doves with no prior experience with their congeneric rival approached the speaker that was reproducing its songs. A similar result was found in the pink pigeon in Mauritius, where males were not able to discriminate between their own species' song and the song of an invasive congener (Wolfenden *et al.*, 2015). Based on the experiments presented in the current study, it appears that male emerald-spotted wood-doves are able to discriminate between their own species' song and the song of the congeneric blue-spotted wood-dove, and do not respond to the latter's songs. It is worth mentioning that the savannah *Turtur* species in Lake Mburo NP often live in much higher densities, with smaller territories, than the forest doves examined in the studies described above. Hence, chance interactions are likely more common between the savannah species, with a reasonably high probability that individual emerald-spotted and blue-spotted wood-doves are spotted close to each other (< 50 m), which was never observed for the pair of forest wood dove species in Ghana.

Another reason for the lack of interspecific interaction in this study could be related to differences in the availability of food resources. In general, the role of seed supply as a factor limiting the numbers of granivorous birds in Africa has been disputed. It is, however, more likely that any such limitations would be found in the Sahel zone, where seed production is quite variable and conditions for collecting food more severe (Zwarts, Bijlsma & Kamp, 2023a). During the doves' breeding period in the savannah, food resources are abundant and are likely not a limiting factor (Lack, 1987), as the primary above-ground production of herbaceous vegetation increases by up to 10 times compared to the Sahel (Zwarts *et al.*, 2023b, 2023a). Hence, interspecific territoriality driven by food resource defence would make little sense from an 'economic' perspective (Brown, 1964), and there should not be evolutionary pressure to chase individuals of related species. The fact that the breeding seasons do not overlap should only weaken such competition further. However, in this scenario it is difficult to identify which factor might be the cause and which the effect.

McCarthy (2006a) suggested that emerald-spotted and blue-spotted wood-doves may hybridise. Hence, in sympatric populations, males of both species might—at least in theory—compete with each other for females (de Kort, 2000; de Kort et al., 2002). Although we did not test females directly, our results seem to contradict this suggestion. If the blue-spotted wood-dove males were a potential threat to co-opt females, we would expect that they would be chased if they sing too close to an emerald-spotted wood-dove's songpost. Moreover, the mentioned hybridisation was observed in captivity (Brickell, 2005) and thus may have no or only limited relevance to natural conditions. To be sure, the missing puzzle piece is knowledge of how females of these two species respond to the song of male congeners. Within their preferred habitats, *Turtur* doves are all quite common across Africa, but their breeding biology, as well as courtship behaviour, is very poorly known. For example, during this study on the emerald-spotted wood-dove, we discovered that males produce previously undescribed songs that have a remarkably different structure compared to the typical ones (own unpublished data). We observed such behaviour rarely, and only when a female was present close to the male. It is thus very likely that male-female interactions within a species are facilitated by another type of acoustic signal used only in this context. A further role is almost certainly played by differences in the colouration of spots on the wings (Baptista *et al.*, 2020a, 2020b), which might be crucial when birds are in close proximity.

What is important for recognition of conspecific song?

Male emerald-spotted wood-doves obviously discriminated between their own species' song and that of a congener. For a human observer, the songs of these two species seem to be very similar, but there are remarkably consistent differences regarding song duration, number and the temporal organisation of notes, and frequency (own unpublished data). The largest difference concerns the temporal organisation of notes as, on average, the song durations of both species are very similar, with those of the emerald-spotted wood-dove being slightly longer (95%CI: 12.0–12.5 s vs. 11.5–11.8 s) with a much higher number of notes (95%CI: 29.4–30.5 vs. 20.0–21.6) (see Figure 1). The primary functions of song (rival deterrence and mate attraction) can be fulfilled only if the song communicates information, which must, above all else, be species-specific (Catchpole & Slater, 2008). Hence, our result is not anything really unexpected; several species pairs, like the doves studied here, have been found to efficiently discriminate their own signals despite superficial biological similarity (reviewed in Catchpole & Slater, 2008). The more important finding is that we cannot confirm that either of the two distinguishable parts of the dove's song carry more species-specific information than the other. The observed differences in the characteristics of the beginning and end parts of the song, in particular in their amplitude, made it tempting to hypothesise that the louder, further-carrying final part might carry species-specific information. However, our experiments demonstrated that recognition is not that simple; it does not appear to be the case that, for example, the final part of the song is used to identify the species, and the initial part is used to identify the individual, their motivation, or something else. It is worth noting that we were playing songs at a distance that allowed—to the best of our knowledge—both parts of the song to be easily heard by the focal bird. This hypothesis is supported by the fact that they sometimes flew toward the speaker before the whole song had ended, i.e., after only the first few syllables of the quieter beginning part. The response was, therefore, not limited by what they could hear but by what they could recognise as species-specific.

Earlier experimental studies on a species' recognition of its own song led to two main conclusions. First, the song features responsible for the recognition of other members of the same species are typically those that are characterised by the least amount of between-individual variation. Second, this can involve different song features among species (Marler, 1960; Becker, 1982), such as the structure of notes, song syntax, timing, or frequency (review in Catchpole & Slater, 2008). However, unlike the experiments conducted by J-C. Brémond (discussed in Catchpole & Slater, 2008), here we did not test the functionality of different song components in coding species specificity. We only tested if either of the two main song parts are responsible for species recognition.

This result is very interesting for several reasons. First, the experiment was designed so that the focal male would be in close proximity to the source of playback (25–35 m). However, the song of both emerald-spotted and blue-spotted wood-doves can be detected by a human observer from a much larger distance, 200–250 m (personal observation). We do not know exactly what the hearing range of doves is, but in some experiments, we observed males returning to the speaker after taking a position in the distance exceeding, in some cases, 100–120 m away (own unpublished data). Although the initial part of the song of the emerald-spotted wood-dove is around 12 dB lower in intensity, on average, than the final part, the latter part is nonetheless generally of low amplitude (average 68 dBA at 1 m). Therefore, it would seem to be impossible for birds to recognise the structural details of the initial song part (see Figure 1) or even detect its presence from 100 m away. Therefore, regardless of our results, when male emerald-spotted wood-doves are listening to birds singing from a greater distance, it is likely they have to make decisions based on the information encoded solely in the final part of the song.

Why, then, do *Turtur* dove songs have two parts and why, among other features, do they differ in amplitude? Songs consisting of two acoustically distinct parts are not uncommon in birds. They can be found in learning Oscines, as, for example, in many Old World buntings, Sylviid warblers, or thrushes. In some cases, such differences in song structure are explained by dialect variations (e.g., in yellowhammer, *Emberiza citrinella* (Diblíková *et al.*, 2019)), while in others, it seems to be related to the intentionally limited range of part of the signal, for example, in the European blackbird, *Turdus merula* (Dabelsteen & Pedersen, 1993). However, it can be dangerous to presume links between different song structures and a single, specific function; this kind of work always requires direct experimental testing. For example, in a close relative of the yellowhammer, the ortolan bunting (*Emberiza hortulana*), the final part of the song was for decades named a "dialect" indicator, with the assumption that it enables the recognition of male origin (i.e., the place where he learned signals). However, experiments testing the response of males to full, foreign, and mixed songs from different dialect areas revealed that the ortolan buntings' perception of locality is more complicated than the opinion of human observers (Osiejuk, Ratyńska & Dale, 2007; Osiejuk, Bielecka & Skierczyński, 2012).

Because the two-part song phrase structure is common to all five *Turtur* species, it is probably a phylogenetically old trait. We hypothesise that differentiating song amplitude within a song allows the singer to limit signal transmission to particular receivers, presumably to his own mate and potential rivals. With respect to species-identity coding, the situation is asymmetrical. If a sender is always singing low- and high-amplitude parts of a song (which in the studied species is the general rule), a closer receiver always receives the information encoded in both parts, but a receiver at a distance would miss the low-amplitude part of the song. However, such a perspective again suggests that there is no need to code species identity in the low-amplitude initial part. The most probable scenario, then, is that the louder final part of the emerald-spotted wood-dove song serves for both mate attraction and rival deterrence, while the softer initial part of the song is a more directed signal aimed at a female (mate or potential mate) close to the sender. In such a case, we would expect that the initial song part is more likely to be individually specific or to reveal the characteristics of a male that are more important to potential mates rather than to a rival.

Conclusions

Even though they live in sympatry with the congeneric blue-spotted wood-dove, emerald-spotted wood-doves responded strongly (i.e., approached the speaker) only when they were confronted with the entire song of their own species. This experiment revealed that our initial assumption—that the features conveying species identity are solely in the final, louder part of the song—was incorrect, as both parts of the song were necessary to evoke a full response. Based on our results, it is impossible to assign species-specific coding to any part of the song of the emerald-spotted wood-dove; however, in natural conditions, the louder final part of the song phrase should propagate much further than the initial part. We found no evidence of serious interspecific interactions or interspecific territoriality between males of these two species—or, at a minimum, that the more abundant emerald-spotted wood-dove defends its territory against its less-abundant congener—in the Ugandan savannah.

Author contributions

TSO conceived this project; MN, PS, LZ, MB and TSO collected data; MN, PS, LZ and TSO performed and analysed playback experiments; MN and TSO performed the statistical analyses and drafted the manuscript and all authors contributed to the editing of the manuscript; PS was responsible for collecting all permits and contact with local authorities.

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Tables and figures

Table 1. Principal component loadings for emerald spotted wood-dove responses to playback of conspecific, congeneric, two mixed and control songs. Kaiser-Meier-Olkin = 0.74, Bartlett's test of sphericity $\chi^2 = 215.826$, $P < 0.001$.

Statistics and original response variables	PC1 - Approaching	PC2 - Vocal response
Eigenvalue	3.169	1.717
% of variance	45.27	24.53
Cumulative %	45.27	69.80
First flight towards speaker (s)	**-0.76**	-0.12
Closest distance (m)	**-0.90**	-0.16
Time ≤ 10 m to speaker (s)	**0.82**	-0.19
Flights during playback	**0.85**	-0.17
Flights after playback	0.53	-0.37
Songs during playback	-0.14	**0.86**
Songs after playback	0.10	**0.89**

Table 2. Numbers of males which approached the speaker in all treatments of the experiment.

Treatment	Number of males		% males approaching speaker
	not approaching	approaching	
TC	1	13	92.9
TC-TA	7	9	56.2
TA-TC	10	7	41.2
TA	9	5	35.7
SC	10	2	20.0

Table 3. Comparison of original response variables (means ± se) for the emerald-spotted wood-doves males responding to playback of conspecific (TC), congeneric (TA), two mixed (TC-TA and TA-TC) and control (SC) songs.

Original response variables	Treatment, *x* ± se				
	TC	TC-TA	TA-TC	TA	SC
First flight towards speaker (s)	62 ± 22.6	284 ± 59.6	327 ± 56.9	220 ± 58.2	374 ± 68.4
Closest distance (m)	6.1 ± 1.80	24.6 ± 3.86	27 ± 3.3	25 ± 3.7	33 ± 2.1
Time ≤ 10 m to speaker (s)	273 ± 57.6	96 ± 45.6	66 ± 44.5	48 ± 42.0	0 ± 0
Flights during playback	6.8 ± 1.50	2.7 ± 0.94	1.5 ± 0.62	1.7 ± 0.64	0.4 ± 0.15
Flights after playback	1.7 ± 0.59	0.8 ± 0.34	0.2 ± 0.11	2.1 ± 1.54	0.4 ± 0.19
Songs during playback	7.1 ± 1.05	8.2 ± 1.38	7.9 ± 1.25	6.1 ± 1.15	5.8 ± 1.36
Songs after playback	10.0 ± 2.03	8.6 ± 1.43	7.8 ± 1.46	5.8 ± 1.6	4.8 ± 1.70

Fig. 1. Spectrograms illustrating songs used for the playback in five treatments of the experiments: Treatment TC - the emerald-spotted wood-dove (*Turtur chalcospilos*); Treatment TC-TA, mixed song with initial part of the emerald-spotted phrase and final phrase of the blue-spotted wood-dove (*Turtur afer*); Treatment TA-TC, mixed song with initial part of the blue-spotted phrase and the final phrase of the emerald-spotted wood-dove; Treatment SC (control) song of the ring-necked dove (*Streptopelia capicola*). Different colours indicate species.

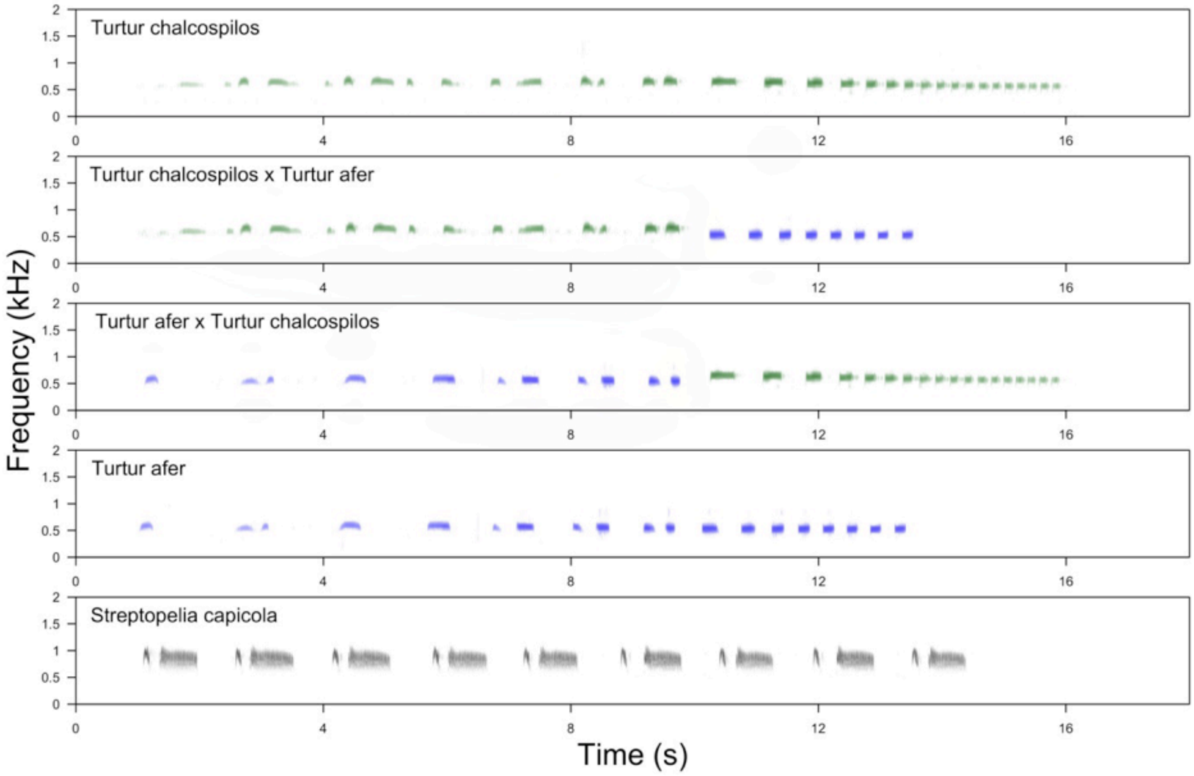


Fig. 2. The emerald-spotted wood-dove males responses to playback of conspecific (TC), mixed (TC-TA, TA-TC), congeneric (TA) and control (SC) songs measured with compound measures of response: PC1-Approaching and PC2-Vocal response.

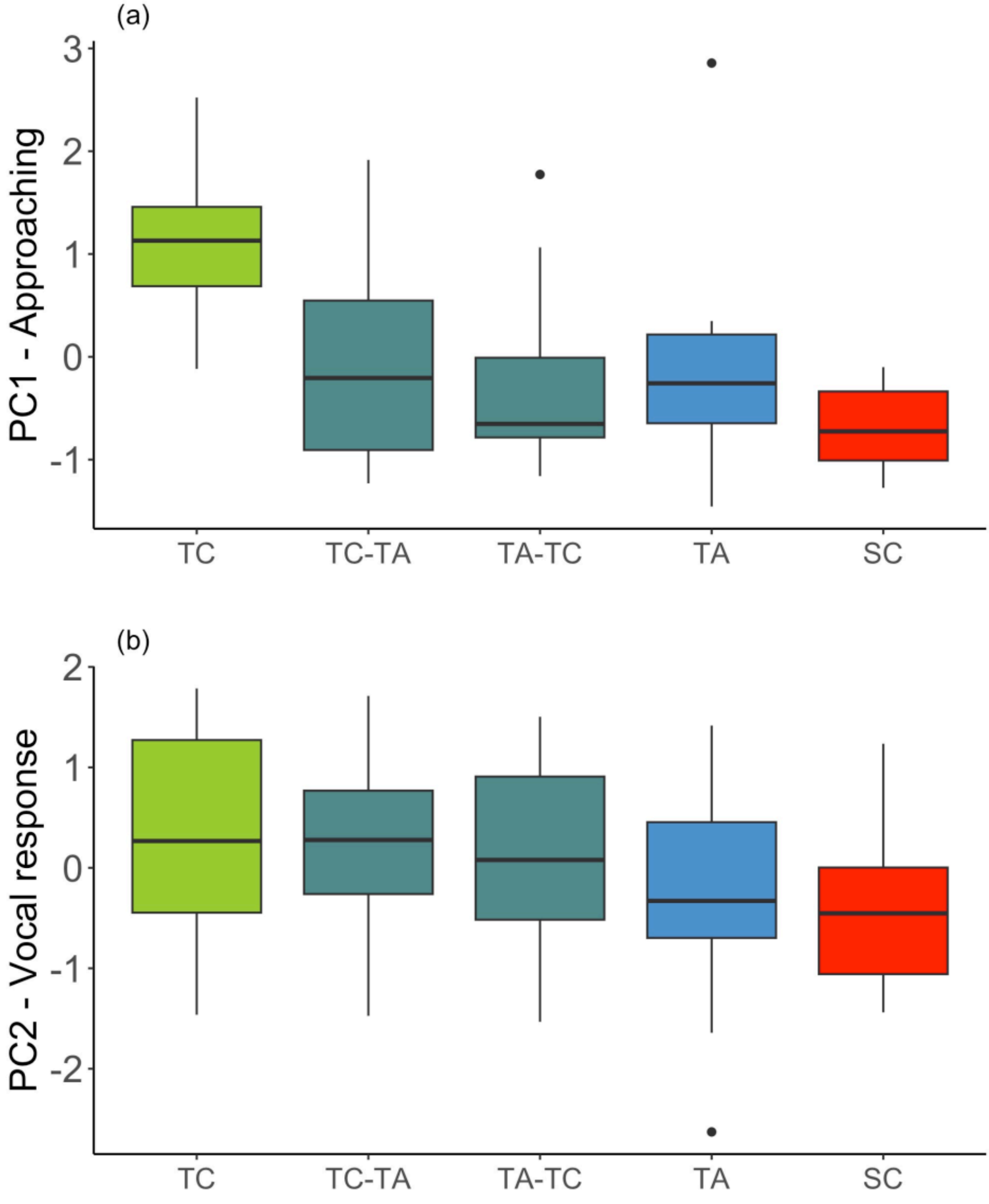
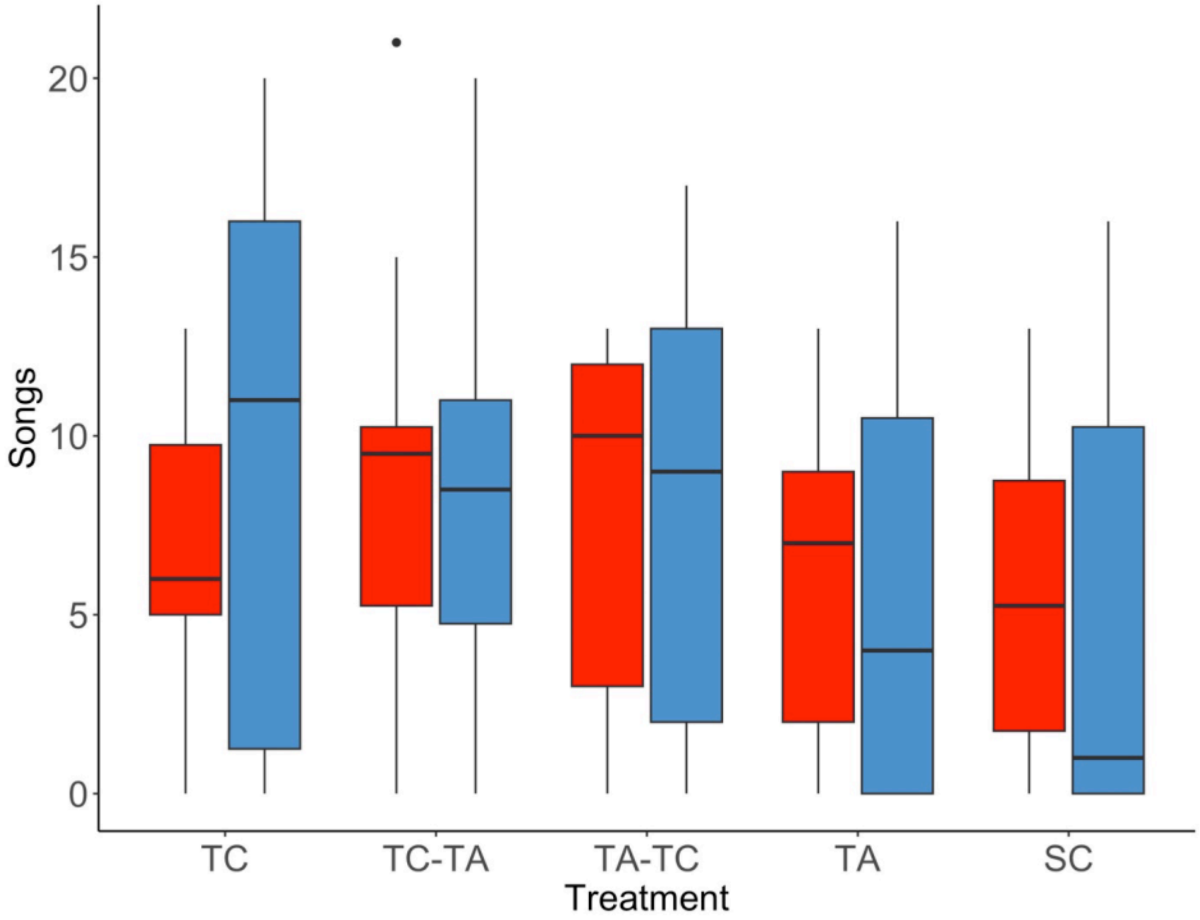


Fig. 3. Number of songs sung during and after playback phase in experiments with the emerald-spotted wood-dove males responding to the playback of conspecific (TC), mixed (TC-TA, TA-TC), congeneric (TA) and control (SC) songs.



CHAPTER 4

Neighbour-stranger discrimination in an African wood dove inhabiting equatorial rainforest

Małgorzata Niśkiewicz, Paweł Szymański, Lia Zampa, Michał Budka and Tomasz S. Osiejuk

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Neighbour–stranger discrimination in an African wood dove inhabiting equatorial rainforest

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We investigated within- and between-individual song variation and song-based neighbour-stranger discrimination in a non-learning bird species, the blue-headed wood-dove (*Turtur brehmeri*), which inhabits lowland rainforests of West and Central Africa. We found that songs of this species are individually specific and have a high potential for use in individual recognition based on the time–frequency pattern of note distribution within song phrases. To test whether these differences affect behaviour, we conducted playback experiments with 19 territorial males. Each male was tested twice, once with the songs of a familiar neighbour and once with the songs of an unfamiliar stranger. We observed that males responded more aggressively to playback of a stranger's songs: they quickly approached close to the speaker and spent more time near it. However, no significant differences between treatments were observed in the vocal responses. In addition, we explored whether responses differed based on the song frequency of the focal male and/or that of the simulated intruder (i.e., playback), as this song parameter is inversely related to body size and could potentially affect males' decisions to respond to other birds. Song frequency parameters (of either the focal male or the simulated intruder) had no effect on the approaching response during playback. However, we found that the pattern of response after playback was significantly affected by the song frequency of the focal male: males with lower-frequency songs stayed closer to the simulated intruder for a longer period of time without singing, while males with higher-frequency songs returned more quickly to their initial song posts and resumed singing. Together, these results depict a consistently strong response to strangers during and after playback that is dependent on a male's self-assessment rather than assessment of a rival's strength based on his song frequency. This work provides the first experimental evidence that doves (Columbidae) can use songs for neighbour-stranger discrimination and respond according to a "dear enemy" scheme that keeps the cost of territory defence at a reasonable level.

Keywords Neighbour–stranger discrimination, Dear enemy phenomenon, Broadcast vocalisation, Song, Columbidae vocalisation, Playback experiment

The ability to recognise one individual from another based on distinctive features has evolved many times across different animal taxa[1] using different signal modalities[2]. In birds, acoustic signals like songs or calls often have individually distinct characteristics that are used for individual recognition in a wide range of contexts, such as parent–offspring recognition, social behaviours (foraging, anti-predator, roosting, etc.), or neighbour-stranger discrimination (hereafter NSD)[3–5]. NSD in birds is strongly supported by research[5,6] and has a well-recognised ecological and evolutionary background[2]. Many bird species defend access to limited resources such as territories, mates, or food, which yields evolutionary benefits in terms of increased fitness. However, defending resources is inherently linked with costs related to signalling, patrolling, and chasing intruders, which may increase energy expenditure, the risk of predation, or even the risk of injury or death caused by a rival. Therefore, territoriality should only be observed if, on average, the benefits from limiting access to resources exceed the costs of their defence[7]. The cost of territorial defence can be reduced by avoiding unnecessary conflict, and one way of doing this is by discriminating between familiar neighbours and unfamiliar strangers[6]. Neighbours do not necessarily constitute a serious threat to a territory holder, whereas the appearance of a stranger carries the risk of territorial insertion, takeover, or interception of a female. Therefore, after a territory's borders are established, the response

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of the territory holder to an intrusion by a familiar neighbour should be less aggressive than the reaction to an intrusion by a stranger. This reduction in aggression toward neighbours has been termed the "dear enemy phenomenon"[8] and has been observed in many territorial birds, as well as mammals, reptiles, amphibians, and insects[6,9,10]. However, a "dear enemy" relationship can be flexible and may evolve with the social and ecological circumstances at hand[11,12]. In certain cases, a neighbour can actually be more threatening than a stranger[13,14]. For example, the song sparrow males adjust behaviour towards neighbours based on their own mate's fertility status and respond stronger to neighbours during periods of female fertility[15].

NSD in a territorial-defence context has been studied primarily in songbirds (Oscines), i.e., birds that acquire a song through social learning during ontogeny (Stoddard 1996). Although it has rarely been directly expressed, the general opinion seems to be that birds who learn their songs should be better at recognition tasks[16]. Indeed, several studies have reported that individual recognition in songbirds is not limited by characteristics of their repertoires (e.g., large or shared between males)[17,18]. Less is known about NSD in non-learning bird species, but increasingly, experimental evidence is arriving from taxa as varied as grouses[19], tyrant flycatchers[20], shearwaters[21], loons[22], gulls[23], woodhoopoes[24], owls[25], and rails[26]. To our knowledge, though, one group of birds that has never been tested with respect to NSD is the family Columbidae. Members of this family are often characterised by stereotyped broadcast signals (referred to as both songs and calls), which may create the impression that there is little space for identity coding. On the other hand, in-depth studies of the mechanisms of vocal production[27,28] and the functions of various characteristics of their voices[29–33] have shown great communicative abilities. Indeed, many pigeons' and doves' songs seem to be aimed at individuals who are far out of sight, and there is evidence that these songs may contain a great deal of individually distinct information[34,35].

Here, we investigated song-based NSD in the blue-headed wood-dove, *Turtur brehmerii* (Columbidae), in its natural environment. We used the term "song" to describe the signal produced by males, serving both to attract mates and to compete with conspecific rivals. Hence, it possesses crucial song characteristics[36]. Blue-headed wood-doves are non-learning, sedentary birds that inhabit lowland rainforest (up to 750 m asl) in western and central sub-Saharan Africa, where visual contact is difficult. The biology of blue-headed wood-doves has not been well characterised, but males are known to defend their territories and occupy the same area for long periods, presumably their whole lives[37]. Males sing spontaneously from treetops, and the song posts of different individuals are typically separated by ≥ 100–150 m. The breeding season is long and likely depends more on food availability than weather conditions[37]. Territorial blue-headed wood-dove males produce a moderately loud song (79–85 dBA SPL at 1 m) consisting of short whistle syllables of increasing rate (Fig. 1). Despite its loudness, the low frequency of the song (with a peak average frequency of 460 Hz; more details in Table S1) and the unmodulated whistles ensure efficient transmission through the forest habitat. Songs can be heard by human observers even from 400 to 500 m (personal observations). The function of this song appears to be equivalent to the function of song in songbirds, i.e., mate attraction and territory defence[38]. Unlike the songs of many songbirds, though, the blue-headed wood-dove song is seemingly very simple in structure.

In this study, we performed the first detailed analysis of basic song parameters in the blue-headed wood-dove and identified which song characteristics might have the potential for identity coding. Then, we used playback to experimentally assess the ability of males of this species to discriminate between the songs of familiar neighbours and unfamiliar strangers, by analysing the behaviour of territory owners during simulated intrusions by neighbours and strangers.

We predicted that if these birds are able to discriminate between neighbours and strangers, we should observe differential responses to the playback of songs from these two groups. Given the sedentary life style and long-term territorialism of blue-headed wood-doves, we expected to observe a stronger response to strangers' songs. Our parallel study on this species demonstrated that the song of a given male has an extremely repeatable frequency, which reflects body size (negative correlation between song pitch and body size) and may potentially influence the response to a rival (in prep.). Therefore, we also performed additional analyses to determine if

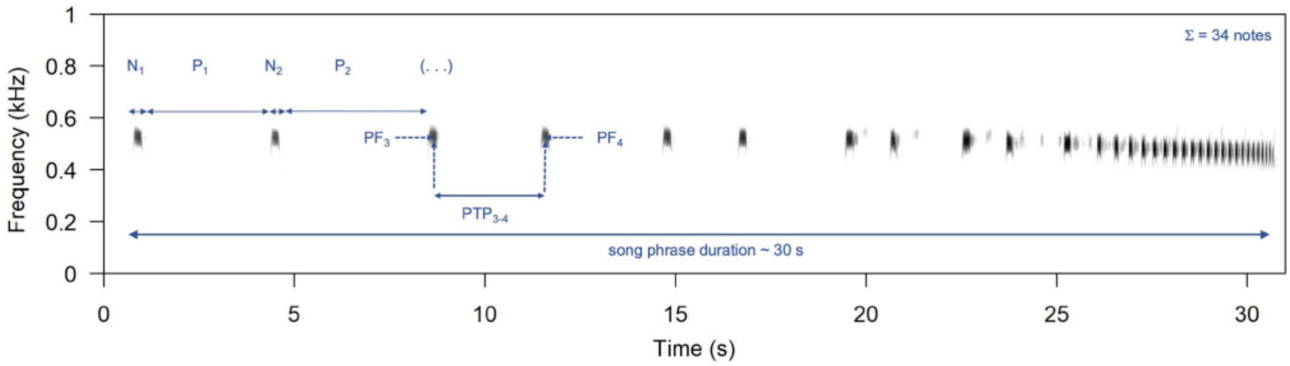

Figure 1. Spectrogram illustrating the song of the blue-headed wood-dove. Letters indicate: N_1, N_2—durations of following notes; P_1, P_2—pauses between following notes, based on manually selected beginning and end of each note; PF_3, PF_4—peak frequency of notes measured with the One-dimensional transformation function of Avisoft SASLab Pro; $PTP_{3–4}$—pauses between syllables measured as time duration between peak frequency of following notes. The figure shows example measurements that were taken for all notes and pauses within each song.

the song frequency of the focal males and/or that of the playback songs affected the responses to the simulated neighbours and strangers.

Methods

Study area

Our study was conducted in Kakum NP in Ghana (5.20–5.40 N, 1.30–1.51 W, altitude 135–250 m asl). The study area was regularly searched and all individuals recorded and mapped. Then, territorial males were observed, recorded, mist-netted, and individually marked (colour rings and LifeTags™, Cellular Tracking Technologies) in February–March 2021, and November 2021. Captured birds of the study species, as well as the co-occurring sibling species (tambourine dove, *Turtur tympanistria*), were weighted and measured in a standard manner. The experimental portion of the study was conducted between 13 November and 4 December 2022. In Kakum NP, the blue-headed wood-dove is a common forest species and prefers patches with high trees close to streams. During the experimental period, birds were vocally active and sang intensively from dawn until 11–12 h, depending on the weather conditions. Males tended to cluster; within a cluster, 2–5 males could typically be heard singing from song posts separated by 100–300 m, from which it was not possible to hear birds from other groups. It seems that in preferred habitats the space was fully filled with territories, and neighbours singing at distances ≥ 100 m from each other did not approach each other.

Recording procedures

We recorded males during preliminary fieldwork and during the experimental part of the study in order to obtain good samples for playback preparation. To determine if the songs of different males have the potential for identity coding, we selected high-quality recordings with at least ten songs of each male. To ensure that we analysed only one individual and his songs at a time, we chose well-spaced males and recorded from a short distance, from a single song post (no movements during recording), and in a single attempt. This limited the sample size to 10 males and 200 songs but allowed for unequivocal identification and characterisation of within-individual song variation. We also followed three individuals with mentioned earlier LifeTags; they were recorded while a second observer simultaneously identified them with a radio receiver, in order to confirm that the songs of individuals were constant in time (subsequent days). Here we present a brief overview of the song characteristics of these birds as they relate to NSD; a more detailed comparison of within- and between-individual song variation in all five *Turtur* species will be the subject of a separate study (in prep.). Recordings were made from the closest distance possible, ca. 20–30 m, with a Sound Devices MixPre-3 or a Marantz PMD 661 MKII recorder and a Sennheiser ME 67/K6 (frequency response 40 Hz to 20 kHz) or Sennheiser MKH-70 directional microphone (frequency response 50 Hz to 20 kHz).

Sound analysis for within-individual comparison

Overall, we carried out slightly enhanced versions of analyses previously used for the tambourine dove, presented in detail by[35]. All analyses were performed using Avisoft SASLab Pro software v. 5.3.00 (Avisoft Bioacoustics Germany[39]) and Raven Pro 1.6.x (Cornell Lab of Ornithology K. Lisa Yang Center for Conservation Bioacoustics[40]). The original recordings were taken with 48 kHz/24 bit sampling rate/quality, but because of SASLab Pro limitations, files in this study were downsized to 16-bit and were band-pass filtered from 150 to 800 Hz with a time-domain filter (FIR) to remove background noise.

First, we characterised whole songs using their duration (s), number of notes, and several frequency derivatives (Hz): peak frequency, lower quartile (25%), mean frequency (50%), upper quartile (75%), spectral centroid, minimum frequency, and maximum frequency (see Table S2 for details; Avisoft-SASLabPro manual[39]). Frequency measurements were taken with the One-dimensional transformation function/Amplitude spectrum (linear) feature of SASLab Pro and with a −18 dB threshold (resolution 0.046 Hz). Song durations and the number of notes were quantified based on selections performed in Raven Pro, which enabled a very restrictive visual inspection of each note. In the next step, we measured the characteristics of notes and pauses between notes within the song: peak frequencies of notes (later PF), note durations (later N), pauses between notes (later P), and pulse-to-pulse durations (later PTP; Fig. 1). The final characteristic was the time between the peak frequencies of adjacent notes. Here, these measurements are presented using a numbering convention with which, for example, PF_1 and PF_2 represent the peak frequency of the first and second notes, while $PTP_{1\text{-}2}$ and $PTP_{2\text{-}3}$ indicate the time between the maximum amplitudes of the first and second notes, and the second and third notes, respectively (see Fig. 1 for illustration). These measurements were derived from selections in Raven Pro, but with settings adjusted separately for frequency and time measurements, which gave the following resolutions: 23.4 Hz × 21.3 ms or 93.8 Hz × 5.33 ms, respectively. These 'within-song' measurements were used to determine if the within-song pattern of note separation is likely to be important for identity coding, as was found for tambourine doves[35].

Experimental protocol

Preparation of song stimuli

To prepare neighbour song stimuli, we recorded singing males 1–3 days before each experiment. Recordings were made from the closest distance possible, ca. 20–30 m, with a Sound Devices MixPre-3 or a Marantz PMD 661 MKII recorder and a Sennheiser ME 67/K6 or Sennheiser MKH-70 directional microphone. Recordings were made during birds' daily active period, usually lasting until 4–5 h after sunrise (sunrise started 5:54–6:02). The exact locations of the singing males were determined with Garmin GPS 65 s receivers. Stranger stimuli were prepared with recordings from the local populations but collected at least 2 km from focal males. These recordings were collected opportunistically on a daily basis. Recordings were taken with 48 kHz/24 bit sampling rate/quality. The playbacks were digitally prepared to 82 ± 2 dB signal pressure level (at 1 m), which is the average natural amplitude of blue-headed wood-dove song (measured with a CHY 650 digital sound level meter; CHY Firemate Co., Ningbo, China). For both the treatment with neighbour and stranger stimuli, the highest quality song was randomly selected from the collection and duplicated 10 times over a period of 5 min (later PLAY) to prepare playback stimuli. All playback stimuli were created with Raven Pro 1.6.x and Avisoft SASLab Pro 5.2.

Experimental design and playback procedure
The playback experiment consisted of two trials, and each male was tested twice, once with the song of a neighbour, and once with the song of a stranger. The order of treatments for an individual was randomised. A neighbour was defined as an individual with a territory bordering that of the subject (not more than 250 m distant), whereas a stranger was defined as a random male from the local population whose territory was more than 2 km distant from the subject. Our observations suggest that a given male rarely sings from song posts separated by more than 250 m (usually less). Hence, a distance of > 2 km was a very conservative criterion for choosing strangers. In other words, the neighbours always stayed within range of their songs and had no other males between their territories. Strangers could not be heard from the focal male territory and there was a space between them and the focal male territory for at least few adjacent territories of other individuals. In case of trials with neighbour song, the one with better quality was chosen. In the case of stranger treatment, the song sample for playback was selected randomly from a list of males that included the geographical position of the recording (> 2 km distance), and recordings of the same individuals were never used more than once. Both treatments (neighbour and stranger stimuli) were performed on the same day between 05:50 and 11:30 (local time). The time between treatments ranged from 19 to 85 min (mean 29 ± 17.3), depending on how quickly the focal birds returned to their pre-stimulus behaviour (i.e., to singing from approximately the same song post and at the same rate as before the trial). In this way, we reduced the influence of potential natural interactions with real rivals between treatments and decreased the chance of testing different individuals (whose identity was checked based on their song pattern after the experiment). We used exclusively unique song recordings of different neighbours and strangers to avoid pseudoreplication[41]. We did not control for the presence of females or the stage of the breeding cycle, but all tested males were highly vocal. During each experiment, we simulated a situation in which a neighbour or stranger male appeared in the territory of the subject with a clear transgression of its boundaries. Before the experiment, one person placed a speaker (JBL Charge 4, Harman International Industries, Stamford, Connecticut, USA), connected to a Marantz PMD 661 MKII player, ca. 2 m above ground and approximately 50 m from the subject. In the second treatment, the loudspeaker was also placed ca. 50 m from the subject, but in a different location within the territory (but also from the side where the neighbour typically sang). Each trial lasted ≥ 11 min and consisted of three parts. In the first part of the trial, the male's song was recorded for at least 1 min before the playback started. The main aim was to record at least 1 or 2 good-quality songs for later comparison with the response. During the second part of the trial (PLAY), the male's behaviour was recorded for 5 min during the playback of 10 songs from the same male, either a neighbour or stranger, depending on the treatment. Song rate of playback reflected well the natural rate of singing.[37] Then, in the POST playback phase of the experiments, the behaviour of males was recorded without any playback for the next 5 min. A similar approach has been used successfully with the same species, in playback experiments investigating recognition of conspecific and congeneric song[42]. Since we knew after the earlier study how the birds reacted to the song of stranger, and that they were completely ignoring the control songs of local tauraco species, we no longer did the control treatment.

During the experiments, we observed the subject's behaviour and recorded the songs he produced (Sound Devices MixPre3+ Sennheiser ME 67/K6 or Sennheiser MKH-70 directional microphones). A second observer was focused on the approaching response and was always placed to have a different viewpoint of the experimental scene. Both observers dictated their observations of bird behaviours, and tracks of recordings were later synchronised to obtain the timing of all activities. Synchronisation was done based on a high-frequency 'ping' sound generated at the beginning and end of the experiment (the 'ping' was far above the frequency of the focal species song and sounded natural, similar to small bird calls). For the vocal response, we characterised the number of songs produced by focal males during the PLAY and POST phases of the experiments. We also measured the latency of the approaching response to the speaker (s), the closest approach to the speaker (m), the amount of time spent within 25 m of the speaker, and the number of flights during the PLAY and POST phases.

Statistical analyses

Individual differences in song
The main aim of the descriptive part of the study was to determine if a comparison of within-and between-individual song variation allows for individual recognition of blue-headed wood-doves, and which song characteristics are likely to serve in identity transmission. For this, we calculated Beecher's information statistic (H_s) for entire song characteristics, first for song duration and frequency components separately and then for all the song characteristics together. Beecher's information statistic (H_s) indicates the amount of information in a system that is available to convey individual identity. The H_s value is proportional to the number of individuals that can potentially be discriminated in a population[43]. All calculations were performed with the *calcHS* function of the R package 'IDmeasurer'[44]. We also calculated H_s for sequences of peak frequencies (PF) and pulse-to-pulse durations (PTP), to determine if the time–frequency pattern of initial notes is male-specific, as was found in the tambourine dove[35]. When the H_s was calculated for multiple variables, its value was presented for all variables at once, regardless of wheter they significantly contributed to distinguish individuals. Before we calculated H_s for multiply variables we also converted them into uncorrelated principal component with *calcPCA* function of the same package.[44] Finally, for comparative purposes, we present the results of discriminant function analyses (DFAs) based on the same dataset to allow readers to associate H_s values with the efficiency of identity assignment

for the study species. We conducted stepwise DFA implemented in IBM SPSS Statistics 28.0.1.0 using the measurements of whole song phrases and sequences of initial notes, which allowed for direct comparison with previous results on the song individuality of the tambourine dove[35].

Playback experiment

Altogether, we measured seven variables that described the responses of males to playback. Because separate tests on the original variables would not be statistically independent or reveal the multivariate character of the response[45], we combined all original variables into two orthogonal principal components (Table 1). For this, we used a principal component analysis procedure (PCA) with varimax rotation and Kaiser normalisation in IBM SPSS Statistics 28.0.1.0. We first ensured that the dataset was suited for such an analysis (Kaiser–Meyer–Olkin measure of sampling adequacy = 0.748, Bartlett test of sphericity = 89.04, $P < 0.001$). The first PC1 variable—Approaching—reflected the rapidity of the approach to the speaker during playback. Lower values of PC1 indicated that males performed many flights, quickly came close to the speaker, and stayed close for a longer time. The second component, PC2—After playback response—reflected the behaviour of the tested males after playback. Higher values of PC2 were associated with a higher number of flights and more songs during the POST phase of the experiments. The interpretation of PC1 is rather straightforward: lower values reflect a stronger response, as approaching a rival is a clear signal of increased aggression and readiness to fight. In the case of PC2, the component was linked with both song and flight activity after the end of the simulated intrusion. The number of flights during the POST period was never high (0–4) in comparison to the PLAY phase (0–13), while the ranges of song numbers produced during the PLAY and POST phases were the same (0–11). Therefore (together with what we observed in detail during the experiments), we would interpret higher values of PC2 as a faster return to the initial activity, i.e., returning to previous song post with a few flights to the previous song post and singing at a normal rate.

We tested for differences in the response to neighbour and stranger songs with generalised linear mixed-effect models (GLMM) using the 'lme4' package of R[46]. Our response variables were PC1 and PC2. The initial models included two main factors: (1) treatment (two levels: neighbour or stranger song), and (2) playback order (two levels: stranger first or stranger second). We included the first-order interaction terms and used male identity as a random effect. In subsequent models, we tested if the frequency of the song of the focal male, as well as that of the song used for playback, affected response strength. We recalculated the mixed models described above with additional independent continuous variables describing focal male frequency, playback frequency, and relative frequency (i.e., difference between focal male and playback frequency).

We checked the models' assumptions using the 'DHARMa' package of R[47] and found that they were not over-dispersed (all $P \geq 0.728$); visual inspection of Q-Q plots confirmed the normality of residuals (Kolmogorov–Smirnov tests $P \geq 0.198$). We present only the best-fitted models, based on the lowest values of AIC. All P values reported are two-tailed.

Ethical note

To our knowledge, the individuals tested in the experiment reflected the population in a representative way with no potential biases resulting from social background, self-selection, habituation, or other factors as indicated in the STRANGE framework[48]. This study was designed and performed in accordance with the ARRIVE guidelines[49]. Our experimental procedure adhered to the ASAB/ABS Guidelines for the care and use of animals (The Ethics Committee (ASAB) and the Animal Care Committee (ABS), 2019) and was approved by all responsible local bodies listed below as well as by the Polish Laboratory Animal Science Association (certificate no. 1952/2015 to TSO, conforming to Directive 2010/63/EU). Our experimental procedures were approved in Ghana by the Forestry Commission (Wildlife Division), permit no. WD/A.185/Vol.13/80.

Statistics and original response variables	PC1–Approaching	PC2–After playback response
Eigenvalue	3.12	1.36
% of variance	44.56	19.37
Cumulative %	44.56	63.93
Songs during playback	0.48	0.31
Songs after playback	0.39	**0.68**
Latency to the first flight (s)	**0.84**	−0.28
Closest distance to speaker (m)	**0.82**	−0.10
Time spent within 25 m to speaker (s)	**−0.75**	−0.23
Flights during playback	**−0.86**	0.11
Flights after playback	−0.21	**0.80**

Table 1. Principal component loadings for blue-headed wood-dove responses to playback of neighbour and stranger songs. Kaiser–Meier–Olkin = 0.748, Bartlett's test of sphericity $\chi^2 = 89.037$, $P < 0.001$. Values that makes a substantial contribution to the overall variance are in bold.

Results

Within- and between-individual song variation

Detailed data of bioacoustics measurements of songs used to estimate identity coding potential are presented in Table S1. Some, but not all, of our measurements of song characteristics—as analysed using 'IDmeasurer' and Beecher's statistic H_s—revealed a potential for identity coding in the study species. The single whole song variables did not seem to convey a large amount of information regarding individual identity (H_s values between 0 and 1.27; Table 2). Likewise, time-related variables (either together or separately) and bandwidth appeared to be completely irrelevant for individual recognition (Table 2). On the other hand, when we calculated H_s for all frequency characteristics together or for all frequency and time characteristics, there was clearly a large potential for identity coding ($H_s = 2.48$). Even higher values of Beecher's statistic were obtained when we analysed values of peak frequencies and pulse-to-pulse duration (see Fig. 2), up to $H_s = 2.51$ for the longest possible sequence.

Next, we applied the methodology from a previous analysis conducted for the tambourine dove[35] to classify songs to different individuals using stepwise DFAs. Based on the most important measurements of whole songs

Song characteristics	H_S for single variables	H_S for pooled time or frequency parameters	H_S for all time and frequency parameters
Time related parameters			
Duration	0	0.79	
Number of notes	0.5		
Frequency parameters			
Peak frequency	1.16	2.02	2.48
Lower quartile	1.06		
Mean frequency	1.07		
Upper quartile	1.07		
Spectral centroid	1.0		
Minimum frequency	1.27		
Maximum frequency	0.76		
Bandwidth	0		

Table 2. Comparison of H_S (Beecher's statistics) calculated for single, grouped (time, frequency) or all (time and frequency) song parameters. H_S indicate the amount of information in a system, the higher value the larger potential for identity coding.

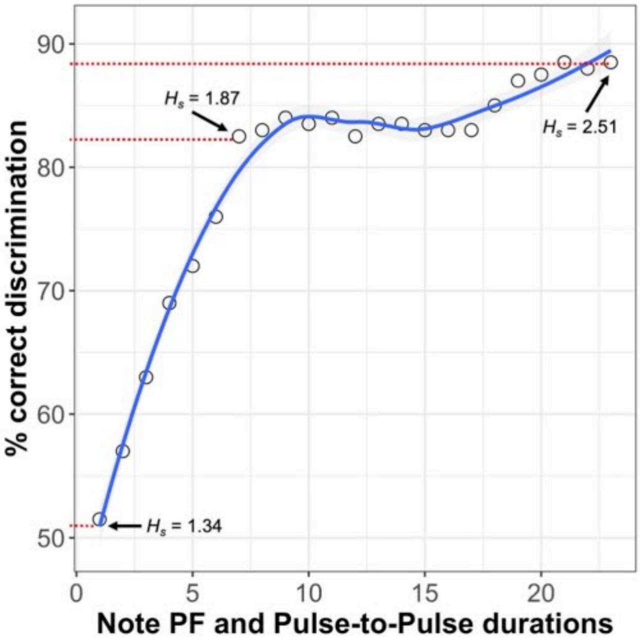

Figure 2. Percentage of correct classification of the blue-headed wood-dove song based on discriminant function analysis (DFA) with note peak frequency (PF) and pulse-to-pulse duration (PTP). Numbers on X-axis indicate sequences analysed with DFAs: 1—$PF_1 + PTP_{1-2}$, 2—$PF_1 + PF_2 + PTP_{1-2} + PTP_{2-3}$ and so on. H_s—Beecher's statistics for the three chosen sequences of PF + PTP. The red dotted lines indicate the data points chosen to showcase the values.

(song duration; number of notes; minimum, maximum, and mean peak frequency), DFA correctly classified individuals in 88.5% of cases in a leave-one-out classification. When we replicated DFAs based on note and pause sequences (PF + PTP)[35], we found that songs were correctly classified 51.5 to 82.5% of cases (sequences contained 1 to 12 notes, which reflected the longest sequence for the tambourine dove measured in[35]. This value increased to 88.5% for analyses of PF_1 to PF_{23} and $PTP_{1\text{-}2}$ to $PTP_{22\text{-}23}$ (Fig. 2). Figure 2 presents values of H_s for a few sequences of PF + PTP measurements, which enables direct comparison between H_s values and the efficiency of discriminant analysis. Both sets of results indicate that differences in the distribution of notes in time create the potential for identity coding.

Response to neighbour and stranger songs

We used playback experiments to determine whether blue-headed wood-dove males ($N = 19$) are able to distinguish between the songs of a familiar neighbour and an unfamiliar stranger. When birds were presented with the two types of playback, we observed significant differences in PC1, the approaching response (Table 3, Fig. 3A), but not in PC2, the after-playback response (Table 4, Fig. 3B). Males responding to stranger playback approached the speaker more quickly, came nearer to it, and stayed close to it for a longer time. The significant Treatment × Order interaction indicated that males responding for the first time to playback of the stranger approached the speaker faster and closer (Table 3). The difference noted in the approaching response also reflected the fact that birds performed more flights in response to strangers' songs during the playback phase of the experiments (Fig. 3A).

	Estimate	SE	z value	Pr(>|z|)
(Intercept)	1.48	0.37	4.02	0.0004
Treatment	−0.84	0.21	−4.01	**0.000897**
Order	−0.45	0.21	−2.17	**0.044**

Table 3. The best (lowest AICc) model explaining variation in PC1-approaching response compound measure of response to playback. The fixed effects in our models were treatment (neighbour or stranger), order of playback and interaction between treatment and order. We included male ID as a random effect. Significant effects are in bold.

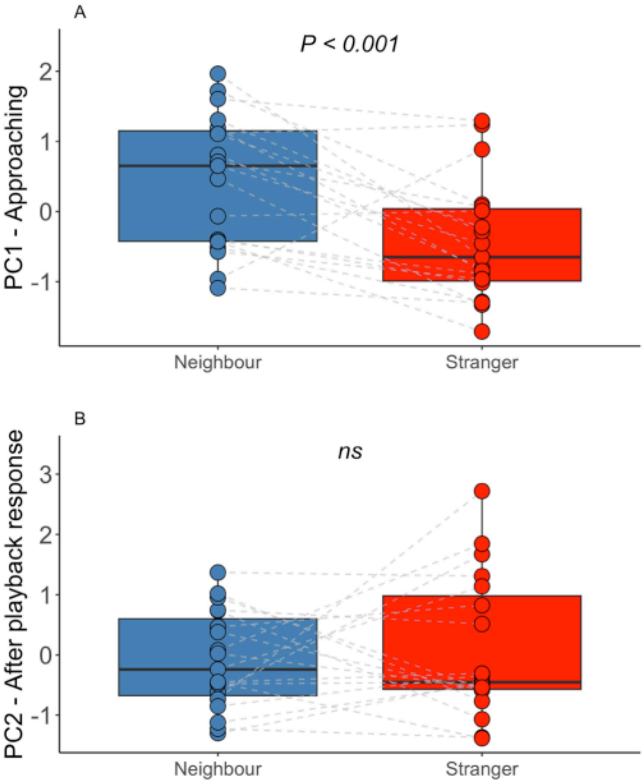

Figure 3. The blue-headed wood-dove male responses to playback of neighbour and stranger songs measured with PC1-approaching and PC2-after playback response compound response measurements ($N = 19$). The dotted lines represent the same tested male responding to neighbour and stranger songs.

	Estimate	SE	z value	Pr(>|z|)
(Intercept)	−0.27	0.50	−0.54	0.597
Treatment	0.18	0.31	0.57	0.576

Table 4. The best (lowest AICc) model explaining variation in PC2-after playback response compound measure of response to playback. The fixed effects in our models were treatment (neighbour or stranger), order of playback and interaction between treatment and order. We included male ID as a random effect.

As mentioned above, we did not control for the frequency of the playback song, which varied among the individuals from whom the songs had been recorded. To investigate if this factor may have had an effect, we built mixed models in which we included the song frequency of focal males, the frequency of the playback, and the relative difference between the two (playback minus focal song pitch). We used mean frequency instead of peak frequency because the mean was less susceptible to deviations due to recording quality, and for the focal males in the experiments, we did not always have many songs to measure (if males did not vocalise very much). However, the correlation between the mean and peak frequency of a given good-quality song was very high (correlation $r = 0.97$, $N = 200$, $P < 0.001$).

Neither the song frequency of the focal male nor that of the playback had a significant effect on the approaching response (PC1), and none of the mixed models that incorporated song frequency were any better (i.e., lower AIC) than the model presented in Table 3. In the case of the PC2 response, we detected a statistically significant effect of the focal male frequency, which greatly improved the initial model (Table 5). Specifically, males singing with a higher frequency had higher values of PC2 responses, meaning that after the playback, they returned more quickly to their previous singing activity and left the vicinity of the speaker. Males who sang lower-frequency songs stayed closer to the speaker for a longer time in the POST phase without singing (Fig. 4). There was a significant Treatment × Order interaction in the final model (Table 5) that indicated that males responding for the first time to the playback returned more quickly to their previous singing activity (higher PC2) than in the second trial with the same individual.

Discussion

By analysing the songs of numerous male blue-headed wood-doves, we discovered that there is a large potential for identity coding in this species. We were then able to demonstrate experimentally that these birds are able to discriminate between the songs of neighbours and strangers. To the best of our knowledge, this study is the first to report NSD in the order Columbiformes and is one of only a few studies showing that NSD occurs in a non-learning bird species.

Individuality of song

We observed that the song of the blue-headed wood-dove is individually specific and that the highest potential for identity coding is found in the unique patterns of note sequences within songs rather than single song characteristics such as frequency or duration. This individual specificity is visually evident from the spectrograms of different individuals (Fig. S1), but more importantly, it was statistically supported by different types of analyses. This result was unsurprising as a similar pattern of individual variation was reported in the closely related tambourine dove[35]. Both species inhabit acoustically similar habitats; hence, similar rules for coding and maximising song transmission should be expected. Despite the similarities between the two species, though, they are easily distinguishable based on different patterns of note timing within the song[37]. In the blue-headed wood-dove, pauses between notes are initially longer and then consistently shortened as the song phrase progresses. In the tambourine dove, pauses in the initial section follow an individually specific pattern and result in the grouping of two or three notes in a distinct series (details in[35]). The two species also differ in size and weight, with the blue-headed wood-dove being much larger (body mass ~ 64% larger) than its congener[50]. It is interesting, however, that the frequencies of their songs are very similar (min–max: 351–562 Hz for blue-headed and 246–551 Hz for tambourine dove; own. unpubl. data), with the smaller species having a slightly lower frequency. Similar

	Estimate	SE	z value	Pr(>|z|)
(Intercept)	−8.69	2.78	−3.12	0.00516
Treatment	−0.61	0.44	−1.37	0.179
Order	−2.15	1.09	−1.98	0.064
Focal male frequency	0.020	0.006	3.28	**0.00372**
Treatment × order	1.50	0.70	2.15	**0.04894**

Table 5. The best (lowest AICc) model explaining variation in PC2-after playback response compound measure of response to playback including song frequency of the focal males. The fixed effects in our models were treatment (neighbour or stranger), order of playback and interaction between treatment and order. The focal male frequency was included in the model as a continuous explanatory variable and male ID as a random effect. Significant effects are in bold.

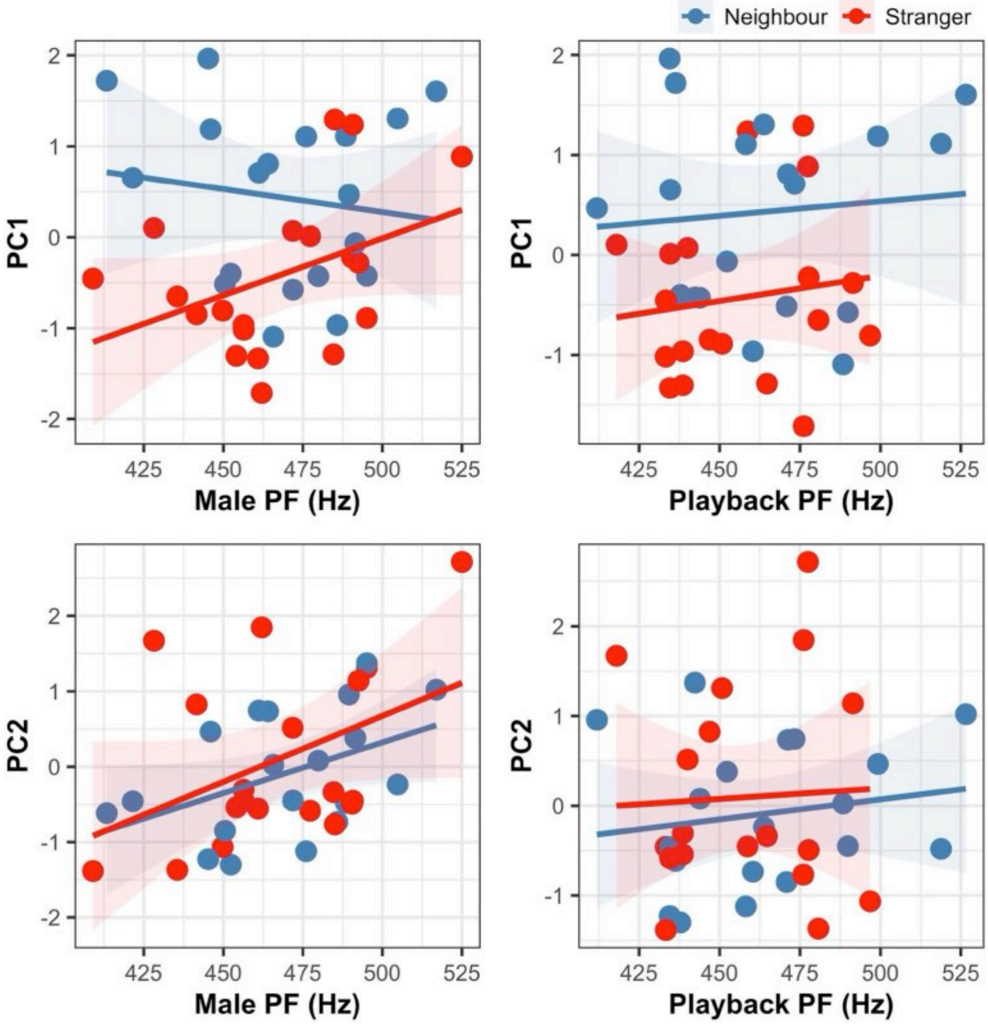

Figure 4. Relationships (linear regression models) between response to playbacks (measured with PC1-approaching and PC2-after playback response) and peak frequencies (PF) of focal male and playback songs. Different colours indicate neighbour and stranger treatments ($N = 19$ males tested twice).

interspecific differences were observed for the pink pigeon *Nesoenas mayeri* and the Madagascan turtle dove *Neosoenas picturata*[51], and overall this suggests that, at least in this respect, their songs may be optimised for habitat propagation[52]. What is most important in the context of this study is that there is undoubtedly a strong acoustic basis for individual recognition in the study species: within-individual song variation is minimal compared to differences between individuals. The overall acoustic simplicity of its song makes the blue-headed wood-dove an ideal model for experimental testing on identity coding using song manipulation and artificially synthesised songs.

Neighbour-stranger discrimination

In blue-headed wood-dove males, the main response axis that differentiated between the response to neighbour and stranger playback was the approach to the speaker. This is quite typical for NSD experiments (e.g.,[53], including those focused on non-passerines[19,26]. When birds approach faster and closer to playback from a potential stranger than from a known neighbour, it is viewed as support for the "dear enemy" hypothesis. This pattern of approach is a good proxy of increased aggression and predicts an escalation to an attack[54].

In the case of the vocal response to the playback of neighbour and stranger songs, it is difficult to detect clear differences, at least at first glance (Table 4, Fig. 3B). Observations of singing males of this species indicate that they adopt a special posture and focus solely on this activity (own unpublished observations). Because song phrases are long, the performance of the song takes time (Table S1). Males rarely produce incomplete songs consisting of only a few (1–4) initial notes, which suggests that once they commit to song production, it is hard for them to stop. Therefore, when a male detects the intrusion of a potential rival from a distance, he most likely has to choose between remaining still and singing from the same position or moving closer to the rival without singing. In this scenario, the number of songs produced during the PLAY phase would be inversely related to the promptness of the approaching response: the faster a male decides to approach, and to try to locate a rival by flying, the less time remains for singing. The males with the strongest response performed their first flight after the first song of the playback and made flights after every, or almost every, song played by the speaker. Hence, approaching during playback is unambiguously the stronger response.

Interpretation of the vocal response is challenging but possible if we keep in mind that it is related to the above-mentioned "mechanics" of responding—namely, it is not possible to sing and approach simultaneously. Analyses that included both approaching and vocal responses revealed more individual variability in the response. Again, such a response pattern is not unusual and has been found in other birds such as the blue grouse (*Dendragapus obscurus*)[19]. Moreover, in bird species that interact with rivals using both songs and calls, the stronger response is often connected with a switch from singing (longer vocalisation) to calling (shorter vocalisation)[20,53].

If focal males maintain the same level of singing during playback as before, it essentially guarantees that they are not moving. In this case, they are still signalling territory ownership but not escalating the conflict. It is less straightforward to decode the meaning of observations in which a male ceased singing but did not approach. Such behaviour could be interpreted as a more cautious response, for example, toward a rival perceived as too strong to respond by approaching[55]. However, it could also be interpreted as a response to a rival viewed as threatening enough to merit attention but not so much that he must be chased away. In practice, when experimenting in tropical forests, we must also always consider the possibility that the focal male did approach the speaker, but we overlooked the flight. We hope that we had no such cases here as the experiments were conducted by two experienced observers who have worked previously with this study species. Regardless, it is also extremely important to link the vocal response during playback with the location of singing after playback. Here, our principal component analysis enabled us to extract variables related to approaching (PC1) and to the behaviour (both vocal and movement) after playback (PC2). Although we did not find a significant effect of the treatment or the order of playbacks on PC2 (Table 4), the addition to the model of the song frequency of the focal male revealed an interesting pattern that sheds light on the factors that shape the response to rivals in this species (Table 5). We found that males who sang lower-frequency songs spent more time during the POST phase singing from a stationary location. In most cases, they were sitting in the last place in which they were found during playback, and it appeared as if the males were waiting to see if the intruder would sing again. Instead, the focal males who sang with a higher frequency came back to their starting positions more quickly and resumed singing from their pre-playback song posts.

To interpret these results, we need to know what factors affect song frequency and what kind of information it may carry. Between-species comparisons usually generate negative correlations between body size and the frequency[56]. This appears to be a general rule for various animal taxa, despite subtle differences related to the evolutionary history of taxa or how measurements of both, body size and song frequency were taken.[57,58] This is explained by the theoretical prediction that larger-sized structures for vibrating and resonating are more effectively producing and coupling lower frequencies to the medium[2]. Theoretically, this pattern of body size–signal pitch allometry should be apparent also within a species. However, it is much less supported by research; some studies found the expected negative correlation, while other did not or even identified a reversed relationship (reviewed in[36,59]). Likely, small songbirds, with their pronounced vocal abilities and the relatively small variability in adult body size, may invalidate this size-pitch relationship . Nevertheless, even for songbirds, when body size parameters correlate negatively with song pitch, the frequency of playback stimuli affects birds' responses. In an experiment on willow warblers (*Phylloscopus trochilus*), males responding to relatively low-pitched songs (compared to those of the subject) stayed farther away from the speaker but responded more actively[59]. This pattern was explained as reflecting the conflicting motivation of the tested males, who were threatened by the low-pitch songs of larger intruders but still wanted to chase off an intimidating rival. In the blue-headed wood-dove, songs are usually produced with a fixed frequency within individuals, and larger males produce lower-frequency songs (own unpublished data). Here, the song frequency used in playback had no effect on either the PC1 or PC2 responses, which indicates that the primary factor shaping the response to a perceived intrusion is the identity of its source (neighbour vs stranger). However, the behaviour of males after playback was significantly affected by their own song frequency (Table 5, Fig. 4). The pattern we found—males with lower-pitch song stayed closer to the speaker for longer without singing—suggests that larger males may have been more motivated to locate a rival and initiate a physical contest. Instead, males with higher-pitch songs quickly returned to their initial song posts after the playback stopped, and started singing again. It was interesting that we did not detect any effect of the playback song pitch per se or the relative difference in pitch (playback minus focal song pitch). This suggests that, when it comes to making decisions about the intensity of defence, the self-assessment of the territory holder is very important. However further experiments with manipulation of rivals' song pitch are necessary for making general conclusions.

Because our experiment was designed primarily to test NSD, not the effect of song frequency, we did not choose songs for playback based on their frequency. For the neighbour treatment, such a choice would be inherently limited by the number of neighbours (and their song frequencies), while for the stranger treatment, a wider range of song frequency variation would be possible (we had a surplus of strangers recorded). In the future, it would be interesting to conduct further experiments that manipulate song frequency so that experimental males are presented with intruders singing with the minimal or maximal frequency found in this species. Such an approach should enable the direct assessment of the function of song frequency in an agonistic context.

Conclusions

Our results are consistent with Fisher's "dear enemy phenomenon"[8] and suggest that blue-headed wood-doves use NSD to keep the cost of territorial defence at a reasonable level. Everything that is currently known about territoriality in this species, including our own observations and recordings in the same locations (between Jan

2019 and Dec 2022), supports a strong attachment to a site, likely life-long (own unpublished data). Such long-term territory tenure in birds is often based on well-established relations with neighbours, for which individual recognition is fundamental[60]. In this context, a strong approaching response to a stranger is not surprising, as this kind of intruder would constitute a stronger threat to the territory holder. Our findings suggest that, as in many songbirds[38], blue-headed wood-doves base their initial decisions about territory defence on song, and tune their response using additional information about rival identity from acoustic stimuli. Extraction of information on body size, based on the comparison of the own and rival song pitch, seems to be possible but must be proven in a separate experiment.

Data availability

The datasets analysed during the current study are available from the corresponding author on reasonable request (Tomasz S. Osiejuk, email: osiejuk@amu.edu.pl).

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Author contributions

T.S.O. conceived this project; M.N., P.S., L.Z., M.B. and T.S.O. collected data and performed experiments. M.N. and T.S.O. performed the bioacoustics and statistical analyses; P.S. was responsible for collecting all permits and contact with local authorities; M.N. and T.S.O. drafted the manuscript and all authors contributed to the editing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Table S1. Summary of the results of bioacoustics measurements (mean±sd) for 10 individuals used for the evaluation of the potential of identity coding.

Male (N song sample)	Song duration (s)	Number of notes	Peak frequency (Hz)	Lower frequency quartile (Hz)	Mean frequency (Hz)	Upper frequency quartile (Hz)	Spectral centroid (Hz)	Minimum frequency (Hz)	Maximum frequency (Hz)	Bandwidth (Hz)
1 (17)	15.6±1.16	30±0.8	440±4.8	432±1.7	443±3.3	472±47.6	453±28.8	417±2.6	469±5.6	52±6.8
2 (13)	12.9±1.46	27±1.8	489±12.8	467±4.5	487±4.2	505±4.2	476±4.1	440±6.6	525±4.7	85±7.5
3 (33)	18.0±1.04	32±0.7	482±5.0	465±5.1	482±2.8	495±5.5	471±4.8	450±4.7	518±7.3	68±8.3
4 (15)	18.4±1.03	27±1.2	498±13.1	481±5.7	495±6.7	507±7.1	491±5.5	458±4.1	523±6.4	66±6.4
5 (21)	14.6±1.62	25±0.9	459±6.4	443±6.4	442±2.6	458±2.5	455±2.0	424±3.1	488±5.1	65±5.7
6 (14)	16.4±1.47	25±0.8	463±6.5	445±1.8	461±1.7	461±1.7	457±2.0	422±1.3	486±2.6	64±3.1
7 (30)	13.8±2.41	25±0.9	443±6.4	431±1.7	444±3.0	454±4.9	444±2.8	412±3.4	471±6.8	59±8.3
8 (10)	18.2±1.68	29±1.5	429±8.2	415±1.7	429±1.9	440±1.6	425±3.7	398±2.0	456±3.3	57±3.8
9 (17)	18.4±2.03	31±1.1	477±6.9	471±3.7	485±5.8	502±8.4	483±6.3	483±6.3	531±22.8	85±24.1
10 (30)	15.9±1.1	27±0.7	423±6.0	414±3.1	426±3.3	441±3.7	429±3.1	392±3.2	459±6.9	67±6.9
Summary of the entire dataset (10 males, 200 songs)										
Grand mean±sd	16.2±2.02	28±2.4	460±25.8	447±23.8	461±25.3	476±25.5	458±21.9	426±22.1	493±29.3	67±10.1
Min–Max	8.5–23.6	23–33	413–520	410–491	421–509	434–625	419–525	388–463	452–597	43–154

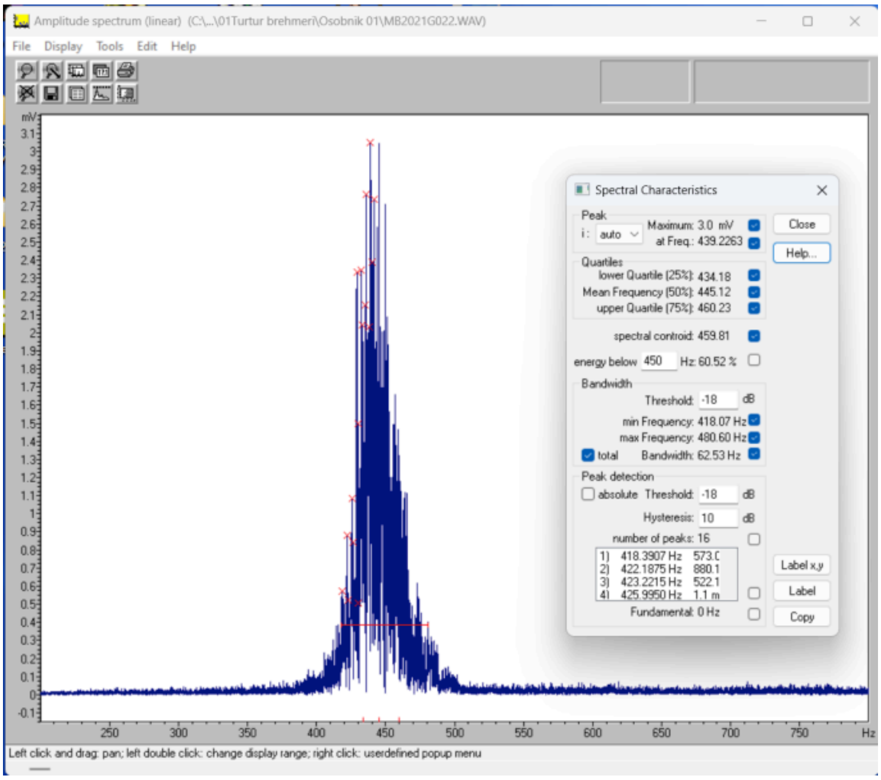
Table S2. Definitions and abbreviations.

Characteristics of song	Abbreviation (if used)	Definition
Note	N or N_x where subscript reflects number of note within song phrase	a continuous line on a spectrogram, which is also referred to as an element; called element; in the case of the studied species, notes are heard as separate sounds, hence, are also equivalent to syllables in other species
Pause	P or P_x (as above)	duration of time between neighbouring notes;
Song phrase		the entire sequence of notes building the song, usually repeated with very stereotyped manner and with gaps between phrases much longer than between notes within phrases
Duration (s)		total time of the song phrase, i.e. from the beginning of the first note to the end of the last note
Number of notes		total number of notes within song phrase
Peak frequency of notes (Hz)	PF or PF_x (as above)	the frequency of the maximum amplitude of a single note spectrum
Pulse-to-pulse duration (s)	PTP or PTP_{x-y} (as above)	time between peak frequencies of adjacent notes

Frequency spectrum characteristics of the entire song phrases[1]

Peak frequency (Hz)	the frequency of the maximum amplitude of the spectrum (where spectrum is measured for the whole song phrase)
Lower quartile (Hz)	the frequency below which 25% of the total energy of the spectrum is located
Mean frequency (Hz)	the frequency below which 50% of the total energy of the spectrum is located
Upper quartile (Hz)	the frequency below which 75% of the total energy of the spectrum is located
Spectral centroid (Hz)	the weighted mean of the spectrum with the magnitudes as the weights
Minimum frequency, Maximum frequency (Hz)	frequencies at which the amplitude falls short of the maximum of the spectrum minus the threshold; the threshold used in our measurements was set to -18 dB (see figure below)
Bandwidth (Hz)	the difference between the maximum and minimum frequency

[1] More details presented here: http://www.avisoft.com/Help/SASLab/menu_curve_display_spectral_characteristics.htm



Peak
i: auto
Maximum: 3.0 mV ☑
at Freq.: 439.2263 ☑

Quartiles
lower Quartile (25%): 434.18 ☑
Mean Frequency (50%): 445.12 ☑
upper Quartile (75%): 460.23 ☑

spectral contoid: 459.81 ☑

energy below 450 Hz: 60.52 % ☐

Bandwidth
Threshold: -18 dB
min Frequency: 418.07 Hz ☑
max Frequency: 480.60 Hz ☑
☑ total Bandwidth: 62.53 Hz ☑

Peak detection
☐ absolute Threshold: -18 dB
Hysteresis: 10 dB
number of peaks: 16 ☐

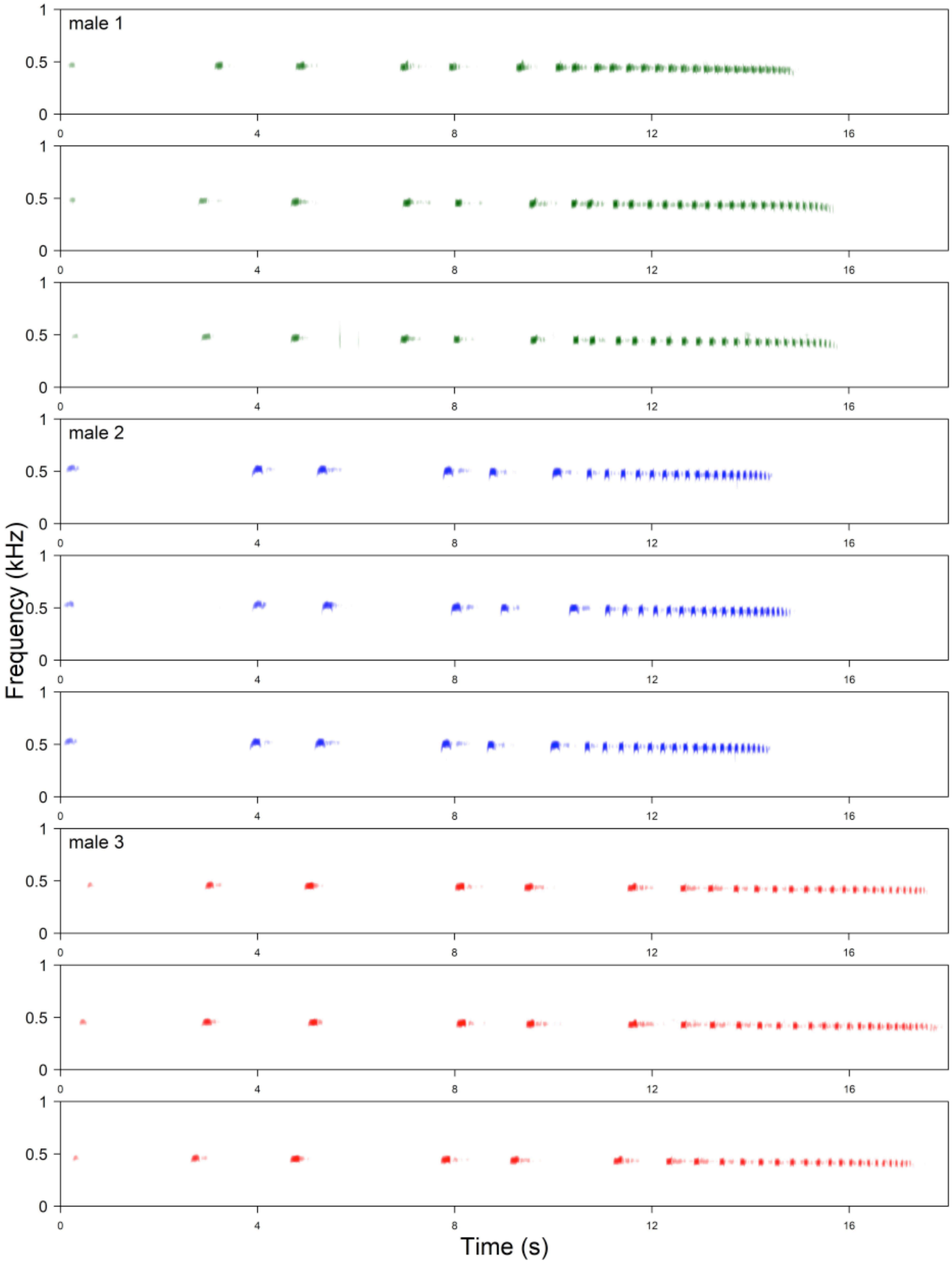
```
1) 418.3907 Hz  573.0
2) 422.1875 Hz  880.1
3) 423.2215 Hz  522.1
4) 425.9950 Hz  1.1 m
```

☐
Fundamental: 0 Hz ☐

Left click and drag: pan; left double click: change display range; right click: userdefined popup menu

The amplitude spectrum window illustrates frequency spectrum measurements taken in Avisoft SAS Lab Pro.

Figure S1. Spectrograms illustrating songs of three different blue-headed wood-dove males (colours indicate different individuals for easier comparison of individual differences).



CONCLUSIONS AND PERSPECTIVES

Recognition of species and individuals within species in the genus *Turtur*

Processes of species recognition, and subsequently of an individual within a species through singing, are particularly important for birds, especially in the context of attracting a breeding mate and deciding how to defend a territory (Mateo, 2004). However, most of these studies focused on songbirds (Oscines), which learn song production, leaving some taxa of Nonpasseriformes, in particular, utterly unexplored in this respect. In my dissertation, I wanted to shed more light on these aspects of communication in one of the nonlearning taxa, namely doves. I have chosen a small group of wood doves, forming five species genus Turtur, endemic to Africa.

Turtur doves possess a unique characteristic among doves: they have long song phrases. While other doves can also produce long bouts, their songs typically consist of short, repeated units. This is evident in doves or pigeons of the genus *Streptopelia* and *Columba* (Winkler et al., 2020). In *Turtur*, the song also consists of repeated notes (syllables), but each may vary slightly or sometimes substantially from the previous. Furthermore, these notes are grouped into initial and final parts, forming a consistent song syntax throughout the entire genus. Their songs consist of low, unmodulated, or very weakly modulated cooing, and this vocalisation is genetically inherited; birds do not need to learn how to produce these vocalisations from other individuals (Baptista et al., 2009). Wood doves live for at least 9 years (Baptista et al., 2020a), and they inhabit their territories for more than one breeding season. They prefer habitats ranging from dense equatorial forest, through miombo to open savannah (Baptista et al., 2020b, 2020a, 2020c, 2020d; Kirwan et al., 2021). Only males of wood doves are known to sing, and in all species except one (*T. tympanistria*), there is no (or very weak) sexual dimorphism in feather colouration. Given all these characteristics, wood doves appear to be an ideal model for studying several factors shaping acoustic signalling in non-learners.

During eight research trips between 2019 and 2023, we collected biometric data, genetic material, and song recordings of all five wood dove species. These data allowed us to describe the songs of wood doves, their habitat preferences, and to check their phylogenetic status (Chapter 1). We found that each species of the genus shared some common features of song, while also had species-specific characteristics, and is individually. We identified these song features that differentiate individuals within each species, further enabling individual recognition. The results presented in Chapter 1 give essential portion of information for formulating more advanced hypotheses on song function as well for designing experiments.

In following, experimental parts of my study I have focused on few selected issues. In the second chapter, we delve into the intricate dynamics of bird song in both intra- and interspecific interactions among African wood doves, with a specific focus on *T. brehmeri* and *T. tympanistria*. Through a series of carefully designed playback experiments, we aimed to unravel whether these species solely respond to their own species' songs or exhibit interspecific territorial behavior, and if such responses are influenced by the presence of congeners. Our findings reveal that both *T. brehmeri* and *T. tympanistria* exhibit strong responses to playback of their own species' songs. However, in regions where they are allopatric, male tambourine doves also respond to congeneric songs, suggesting that the ability to discern between the songs of potential competitors is acquired through interactions and learning. Our study lends support to the misdirected aggression hypothesis, indicating that interspecific territoriality may emerge due to signal similarity. Nonetheless, we propose that on an evolutionary timescale, this ability may serve as an adaptive cognitive tool for resolving competing interests with congeners.

In the next experiment (Chapter 3), we tested a savannah species (*T. chalcospilos*) in a sympatry with another wood dove (*T. afer*). However, we used not only full songs of both congeneric species but also mixed songs containing the initial part of one species and the final one of the second species, and vice versa. Crucially, our results indicate that *T. chalcospilos* possess the ability to discriminate between their own songs and those of congeners, thereby refuting the presence of interspecies territorialism. Additionally, our study failed to identify a single part of the song responsible for coding species specificity, as mixed songs did not significantly enhance responsiveness to playback. These findings contribute to our understanding of interspecific territoriality and the evolution of species-identity coding, prompting further exploration into the complexities of bird communication and social behaviour.

In the final experiment, we explored the intricacies of within- and between-individual song variation and its role in neighbour-stranger discrimination among the blue-headed wood-dove (*T. brehmeri*). Our findings revealed that the songs of this species are individually distinct and hold potential for individual recognition based on the time–frequency pattern of note distribution within song phrases. To examine the behavioural implications of these differences, we conducted playback experiments involving 19 territorial males. Interestingly, we observed that males responded more aggressively to playback of a stranger's songs, approaching the speaker quickly and spending more time near it. However, no significant differences were observed in vocal responses between treatments. Further investigation into whether responses varied based on song frequency parameters yielded intriguing results. While song frequency parameters had no effect on the approaching response during playback, the pattern of response after playback was significantly influenced by the song frequency of the focal male. Males with lower-frequency songs remained closer to the simulated intruder for a longer period without singing, whereas those with higher-frequency songs returned more quickly to their initial song posts and resumed singing. Overall, our study provides compelling evidence that doves (Columbidae) can utilise songs for neighbour-stranger discrimination and respond according to a "dear enemy" scheme (Fisher, 1954; Temeles, 1994), effectively managing the cost of territory defence. These findings shed light on the complex interplay between song variation and social behaviour in non-learning bird species.

Possible future directions

There is a considerable amount of work to be done in wood dove research. We have not been able to explore all aspects of interest during this project, mainly due to insufficient time and independent constraints like COVID 2019 pandemic. Another essential step in the future could be to study species and individual recognition based on song by testing females. Such task is always more demanding in field conditions, especially for work with species without sexual dimorphism. Among wood doves only the T. tympanistria could be sexually recognised without catching and individual marking, hence, it seems to be perfect species – among wood doves – to start with. In case of females, costs of a wrong decision in mating is much higher than for males, thus we may expect that females are even more sensitive for detecting the own species song as well song features related to male quality (Catchpole and Slater, 2008; Searcy and Brenowitz, 1988).

Another important direction of future research could be to know precisely how birds occupy territories for the first time and how interactions between territory owners and newcomers work. We observed the same individuals in the following seasons, in precisely the same places, what with neighbour-stranger discrimination, and bird longevity, allow supposing that occupying a new territory for a young male could be not easy task. In the future studies on male-male interactions it would be necessary to consider negative correlation between body size and song frequency, and its role in signalising strength or resource holding potential of males. The descriptive part of my thesis (Chapter 1) gives a great foundation for any experiments with manipulation of the song structure, which should enable to directly test functions of any song component. I strongly believe Turtur doves as a group, has a great potential for more in depth studies of communication and coding information in signals in particular.

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T.S.O. conceived this project; M.N., P.S., L.Z., M.B. and T.S.O. collected data and performed experiments. M.N. and T.S.O. performed the bioacoustics and statistical analyses; P.S. was responsible for collecting all permits and contact with local authorities; M.N. and T.S.O. drafted the manuscript and all authors contributed to the editing of the manuscript.

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Niśkiewicz M, Szymański P, Herdegen-Radwan M, Zampa L, **Budka M**, Osiejuk TS () "Inter- and intra-specific variation of song in African wood doves – effects of phylogeny, morphology, and habitat", **unpublished manuscript.**

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Niśkiewicz M, Szymański P, **Budka M**, Tomasz Osiejuk (2023) „Response of forest Turtur doves to conspecific and congeneric songs in sympatry and allopatry" **Scientific Reports** (2023) 13:15948 | https://doi.org/10.1038/s41598-023-43035-8

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