Pedro Miguel Filipe Pereira

The role of birdsong and foraging behaviour in mediating the interspecific competition between two sympatric woodland passerine birds: the Robin and the Blackcap

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The role of birdsong and foraging behaviour in mediating the interspecific competition between two sympatric woodland passerine birds: the Robin and the Blackcap

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Pedro Miguel Filipe Pereira

Doctoral thesis in Biosciences (Scientific area of Ecology) supervised by Prof. Dr. Paulo Jorge Gama Mota and Dr. Rui do Nascimento Fazenda Lourenço presented to the Department of Life Sciences of the Faculty of Sciences and Technology of the University of Coimbra

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Cover: European Robin *Erithacus rubecula* (pisco-de-peito-ruivo) by Pedro F. Pereira

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Chapter 2

Pereira PF, Lourenço R, Mota PG. Habitat-shift as potential evidence for competition between two distantly related and widespread European Passerines. Submitted to "Ethology Ecology and Evolution".

Chapter 3

Pereira PF, Almeida L, Lourenço R, Mota PG. Heterospecific aggression over feeding by two sympatric birds differing in territorial behaviour. Submitted to "Ethology".

Chapter 4

Pereira PF, Godinho C, Vila-Viçosa MJ, Mota PG, Lourenço R. 2017. Competitive advantages of the red-billed leiothrix (*Leiothrix lutea*) invading a passerine community in Europe. Biological Invasions 19:1421–1430. doi:10.1007/s10530-016-1364-2

Chapter 5

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Chapter 6

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Contents

Summary

Interspecific competition is an important mechanism contributing to the evolution of species as it can shape the behaviour of individuals, and ultimately affect population dynamics. Species with a long-term coevolution history, which are potential competitors, developed some mechanisms which allow their spatial coexistence, such as resource partitioning or interspecific territoriality. Interspecific competition can occur not only among species with a long-term coevolution, but also between species which have a relatively recent contact, such as the case of native species and species introduced by humans. Despite the relevance of interspecific competition on the evolution of species and structuring of communities, it has been relatively neglected. The lack of knowledge about interspecific competition highlights the need to increase the research on aggression and dominance relationship between distantly related species. Therefore, I decided to conduct a study on competition comparing its intensity between conspecifics and distantly related species. As main study species, I selected two distantly related passerine species, the robin (*Erithacus rubecula*; Family Muscicapidae) and the blackcap (*Sylvia atricapilla*; Family Sylviidae), that are potential competitors for habitat and feeding resources. To analyse the interactions with a recently-introduced species, I also conducted some experiments with the red-billed leiothrix (*Leiothrix lutea*; Family Timaliidae), an exotic species introduced from Asia, which is a potential competitor with the first two species. The main objective of this thesis was to found evidence of competition between these species and, once found, I also tried to understand the ecological and behavioural adaptations that can contribute to reduce it. The detailed objectives were: (1) to analyse similarities in the habitat used by the robin and blackcap in coexistence and in isolation aiming to test the existence of habitat shifting between them; (2) to quantify the levels of aggressiveness in the robin and the blackcap towards conspecifics and heterospecifics, while competing for food; (3) to identify the potential competitive advantages of the red-billed leiothrix during the establishment process in a community of native passerines by collecting morphological and behavioural data; (4) to study the behavioural dominance of red-billed leiothrix over native passerines in a feeding context; (5) to test the use of singing behaviour by the robin and the blackcap as an aggressive signal towards heterospecifics using song playbacks of different species in their natural territories, including native competitors, exotic-competitors and non-competitor species. I found complementary evidences of interspecific competition among the study species. The main results of the thesis were: (a) the occurrence of habitat shifting between

the robin and the blackcap, (b) the relatively high levels of heterospecific aggression between the robin and the blackcap, (c) a high overlap of the morphological niche between the leiothrix and (mainly) the robin, (d) behavioural dominance of the leiothrix over native species in feeding context, and (e) the use of song by the robin and the blackcap as a signal of aggressiveness towards some heterospecifics. These results are relatively novel since: (i) I found only two studies considering the hypothesis of interspecific competition as being in the origin of habitat divergence between distantly related species; (ii) I found a possible advantageous effect of morphological traits of a colonizer species within a bird community which was seldom studied; (iii) to the best of my knowledge, I conducted the first experimental work where clear dominance by an exotic bird species over native rivals was confirmed in a feeding context. The main conclusion of this thesis is that interspecific competition can occur between distantly related species and that it is possible to measure it. By using the robin and the blackcap as models to study interspecific competition, I demonstrated that distantly related species which coevolved and coexist in the same habitats can show important levels of heterospecific aggression – sometimes as intense as among conspecifics – and developed some mechanisms to reduce interspecific competition, such as habitat shift. I also demonstrated that the leiothrix is dominant over native species, such as the robin and the blackcap, which perhaps contributes to justify why it is becoming established so rapidly in Europe. This work highlights (1) the important role of interspecific competition in the use of space between distantly related species that coevolved, and (2) the ability of the native species to deal with a newcomer competing species.

Keywords: aggressiveness, behavioural dominance, competition, distantly related species, feeding behaviour, heterospecifics, invasive species, singing behaviour

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Resumo

A competição interespecífica é um importante mecanismo controlador da evolução das espécies, uma vez que pode moldar o comportamento dos indivíduos e, em última instância, afectar as suas dinâmicas populacionais. Espécies com uma longa história de coevolução, que sejam potenciais competidoras, desenvolveram alguns mecanismos que permitiram a sua coexistência especial, tal como a partição dos recursos ecológicos utilizados e a territorialidade heterospecífica. A competição interespecífica pode ocorrer também entre espécies que iniciaram o contacto há relativamente pouco tempo, como é o caso das espécies nativas e das exóticas introduzidas pelos humanos. Apesar da importância da competição interespecífica na estruturação das comunidades, esta tem sido relativamente pouco estudada. A escassez de conhecimento acerca da competição interespecífica realça a necessidade do aumento do estudo acerca da agressividade e das relações de dominância entre espécies muito distantes do ponto de vista taxonómico. Consequentemente, eu decidi elaborar um estudo acerca da competição comparando a sua intensidade entre conspecíficos e espécies taxonomicamente-distantes. Como principais espécies em estudo, seleccionei duas espécies de aves Passeriformes taxonomicamente-distantes, o pisco-de-peito-ruivo (*Erithacus rubecula*; Familia Muscicapidae) e a toutinegra-de-barrete (*Sylvia atricapilla*; Familia Sylviidae), duas espécies potencialmente competidoras por habitat e alimento. Para analisar as interacções com uma espécie recentemente introduzida, também realizei algumas experiências com o rouxinol do Japão (*Leiothrix lutea*; Familia Timaliidae), uma espécie exótica, nativa da Ásia, e que é um potencial competidor das duas primeiras espécies. O principal objectivo da tese foi procurar provas de competição entre essas espécies. Uma vez encontradas, também tentei compreender as adaptações ecológicas e comportamentais que contribuem para reduzi-la. Os objectivos detalhados foram: (1) analisar as semelhanças no uso de habitat entre o pisco-de-peito-ruivo e a toutinegra-de-barrete em coexistência e isolamento com o objectivo de verificar existência de segregação no uso de habitat; (2) quantificar os níveis de agressividade no pisco-de-peito-ruivo e na toutinegra-de-barrete relativamente aos conspecíficos e heterospecíficos, quando competem por alimento; (3) identificar as vantagens adaptativas potenciais do rouxinol do Japão durante o seu processo de estabelecimento numa comunidade de espécies nativas através do estudo de características morfológicas e comportamentais; (4) estudar a dominância comportamental do rouxinol do Japão sobre espécies nativas de Passeriformes num contexto alimentar; (5) testar o uso do canto do pisco-de-peito-ruivo e da toutinegra-de-barrete como sinal

agressivo para os heterospecíficos utilizando *playbacks* do canto de diferentes espécies nos seus territórios, incluindo um competidor nativo, competidor exótico e não-competidor. Eu obtive provas complementares de competição interespecífica entre as espécies estudadas. Os principais resultados foram: (a) ocorrência de segregação de habitat entre o pisco-de-peito-ruivo e a toutinegra-de-barrete, (b) a obtenção de níveis relativamente altos de agressividade heterospecífica entre essas duas espécies, (c) uma grande sobreposição de nicho morfológico entre o rouxinol do Japão e – principalmente – o pisco-de-peito-ruivo, (d) dominância comportamental do rouxinol do Japão sobre as espécies nativas em contexto alimentar, e (e) o uso do canto pelo pisco-de-peito-ruivo e a toutinegra-de-barrete como sinal agressivo aos heterospecíficos. Estes resultados são relativamente inovadores, uma vez que: (i) encontrei apenas outros dois estudos considerando a hipótese da competição interespecífica como estando na origem da divergência de habitat entre espécies taxonomicamente distantes; (ii) obtive resultados de características morfológicas potencialmente vantajosas de uma espécie colonizadora dentro de uma comunidade de aves, o que constitui um tópico pouco estudado; (iii) do meu conhecimento, esta tese poderá incluir o primeiro estudo experimental onde a clara dominância de uma espécie exótica de ave sobre espécies nativas foi confirmada em contexto alimentar. A principal conclusão desta tese é que a competição interespecífica pode ocorrer entre espécies taxonomicamente distantes e que esta é passível de ser medida. Através do uso do pisco-de-peito-ruivo e da toutinegra-de-barrete como espécies modelo para o estudo da competição interespecífica, demonstrei que espécies distantes que coevoluíram e coexistem nos meus habitats podem apresentar importantes níveis de agressividade heterospecífica – por vezes tão intensa como a que ocorre entre conspecíficos – e desenvolveram alguns mecanismos de modo a evitar a competição interespecífica, como seja a segregação de habitat. Demonstrei também que o rouxinol do Japão é dominante sobre as espécies nativas, tal como o pisco-de-peito-ruivo e a toutinegra-de-barrete, o que poderá contribuir para explicar o seu rápido estabelecimento na Europa. Este trabalho destaca (1) o papel importante da competição pelo uso do espaço entre espécies que coevoluiram, mas que são taxonomicamente distantes e (2) a capacidade das espécies nativas lidarem com uma espécie competidora alienígena.

Palavras chave: agressividade, competição, comportamento alimentar, canto, dominância comportamental, espécies invasoras, espécies taxonomicamente distantes, heterospecíficos

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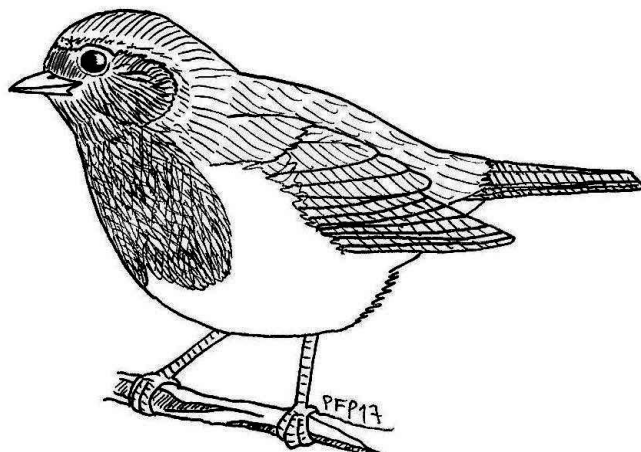
Chapter 1 – General Introduction

In terrestrial ecosystems, competition for resources (such as food or shelter) is susceptible of occurring both within and between animal species (Peiman & Robinson 2010; Losin et al. 2016). In general, the intensity of competition is higher among conspecifics than among heterospecifics as conspecifics often have greater similarity in their morphology, ecological niches and behaviour (Walls 1990; Anderson & Grether 2010). Moreover, such competition intensity can vary spatially or seasonally depending on the similarities of the interacting individuals or the amount and quality of the resource (Cody 1978; Gabor & Jaeger 1995; Arnott & Elwood 2008; Lisičić et al. 2012). Often, some heterospecifics can compete for shelter or feeding resources. While, conspecifics can compete also for nesting sites and for mates.

Coexistence of species with similar ecologies creates a potential of competition between them (Minot 1981; Davies et al. 2007; Lisičić et al. 2012; Losin et al. 2016). Interspecific competition is an important mechanism contributing to the evolution of species as it can shape the behaviour of individuals, and ultimately affect population dynamics within communities by influencing niche occupancy, and patterns of temporal and spatial distribution (Mac Nally 1983; Schoener 1983; Pigot & Tobias 2013). There are two forms of interspecific competition: exploitation, when resources used by one species reduce their availability for competing species (Minot 1981; Petren & Case 1996), or interference, when one species restricts the access to resources to a competing species (Schoener 1983; Walls 1990; Downes & Bauwens 2002; Eccard et al. 2011; Lourenço et al. 2014). The occupation of a limiting number of nest boxes or the consume of a highly season food resource are two examples of exploitation competition as their use (or consume) by a species limits the fitness of the other species (Minot

1981; Schoener 1983). While, direct interference mechanisms include several aggressive interactions such as chemical competition, aggressive behaviour and killing without victim consumption (Lourenço et al. 2014).

Species with a long-term coevolution history developed some mechanisms which allow their spatial coexistence, such as resource partitioning or interspecific territoriality (Leal & Fleishman 2002; Losin et al. 2016). These mechanisms may vary according to the spatial scale of analysis (Wiens 1989; Huston 1999). For example, the degree of habitat specialization and different competitive abilities possessed by each species can allow their coexistence, respectively, at a regional scale and at a more local scale (Kneitel & Chase 2004). In general, species with wide geographical distributions or broad niches, and consequently great genetic variability, are more prone to develop mechanisms to avoid or reduce the competition, such as the ecological and behavioural shifting with their direct competitors (i.e. change some of their ecological or behavioural characteristics as a consequence of a competitive pressure; Connell 1980; Case et al. 2005; Davies et al. 2007). Examples of ecological and behavioural shifting, includes the change of habitat use and the temporal adjustment of bird song according the presence of a competitor (Werner & Hall 1977; Brumm 2006).

Competition for limited resources often generates agonistic interactions between individuals (Ostfeld 1985; Gabor & Jaeger 1995; Duckworth 2006), which can vary according to the overlap of resources used by each opponent (Case & Gilpin 1974; Petren & Case 1996; Grether et al. 2009; Peiman & Robinson 2010). Generally, the willingness to dispute resources depends on a trade-off between the costs and benefits for individuals in obtaining them (Smith 1982). Moreover, it is expected aggressiveness to be more frequent among conspecifics than among heterospecifics. Also, individuals should be less willing to approach heterospecifics than conspecifics, due to greater uncertainty about fighting costs (Peiman & Robinson 2010). The consistency in winning such contests by some individuals against others contributes to establish dominance relationships between them (Drews 1993; Robinson & Terborgh 1995).

When resources are susceptible of being monopolized, individuals may defend territories for exclusive access, which can reduce the costs of disputes (Verner 1977). Territoriality for the exclusive access to space, food or mates is an ecological outcome

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resulting from the aggressive behaviour among neighbours (Maher & Lott 1995; Pyke et al. 1996). In some cases, individuals behave aggressively against conspecifics as well as against heterospecifics which indicates the existence of interspecific territoriality (Walls 1990; Anderson & Grether 2010). Several studies in different taxonomic groups found that the frequency of aggressive behaviour between heterospecifics tends to be higher when individuals defend exclusive territories (i.e. without overlapping with other species) than when they are not territorial (birds: Robinson & Terborgh 1995; fishes: Genner et al. 1999; ants: Tanner & Adler 2009). There are several different kinds of aggressive behaviours, including agonistic displaying or attacking. Territorial birds, in particular, can also display an agonistic intent through their singing behaviour (Searcy & Beecher 2009). Vocal interactions of singing birds can arise between neighbours within their own territorial boundaries or during a territorial intrusion (Naguib 2005). The plasticity of some song types combined with the great mobility of birds allow a relatively fast response after a competitor disturbance (Robinson & Terborgh 1995; Dabelsteen et al. 1997; Hof & Podos 2013). The territorial function of singing behaviour has been studied broadly in conspecific disputes (Naguib 2005; Searcy & Beecher 2009). However, the role of singing behaviour in the context of heterospecific territoriality has been seldom studied (Martin et al. 1996).

Interspecific competition can occur not only among long-term coevolved species, but also between species which have a relatively recent contact, such as the case of native and introduced species. The human-caused introduction of a species in a new environment can lead to even more unpredictable interspecific interactions, depending for example on the ecological overlap between introduced and native species (Petren & Case 1996; Shea & Chesson 2002; Sol et al. 2012). If the introduced species develops a self-sustained population (i.e. without human support) with reproductive success, we can say that the species became established in the new environment (Duncan et al. 2003). During the establishment process, an introduced species can occupy an empty ecological niche without competing with native species, a process designated as "opportunistic hypothesis" (Shea & Chesson 2002; Sol et al. 2012; Batalha et al. 2013). Alternatively, when ecological niches of introduced species and native species overlap, they will often compete for the same resources and thus the establishment of the introduced species is dependent on its superior competitiveness, a process designated as

"competition hypothesis" (Petren & Case 1996; Holway 1999; Foster & Robinson 2007; Sol et al. 2012). The current knowledge on the effects of introduced species in natural habitats (where the impact in native communities should be greater than in highly disturbed habitats) is still limited when compared with humanized habitats, such as farmlands and urban areas (Sol et al. 2012; Martin-Albarracin et al. 2015).

Despite the relevance of interspecific competition on the evolution of species and structuring of communities, it has been relatively neglected by comparison with intraspecific competition (Grether et al. 2009; Peiman & Robinson 2010). The great number of reported cases of interspecific aggression in a wide diversity of taxa suggests it has a high prevalence in nature (salamanders: Walls 1990; passerine birds: Duckworth 2006; rodents: Eccard et al. 2011). Phylogenetically-distant species (from different genus or different families) may engage in aggressive interactions, since they can compete for limited resources (Martin et al. 1996; Duckworth 2006; Freeman 2016). However, most studies have been focused on competition between closely-related species. The lack of knowledge about interspecific competition highlights the need to increase the research on aggression and dominance relationships between distantly related species (Dayan & Simberloff 2005; Grether et al. 2013; Freshwater et al. 2014; Martin & Ghalambor 2014; Freeman 2016). Therefore, I decided to conduct a study on competition comparing its intensity between conspecifics and distantly related species.

Study species

Many insectivorous woodland passerines have been used as model species to study interspecific competition for decades of research (Cody 1978; Minot 1981; Martin et al. 1996; Hansen & Slagsvold 2003; Matyjasiak 2005; Vallin et al. 2012; Reif et al. 2015; Losin et al. 2016). This bird group presents several advantages to study interspecific competition, such as small territories that are often aggressively defended, large scale overlapped distributions, and several ecological similarities between species from different taxonomic families. However, most studies within this subject have been focused on competition between congeneric species, often between hole-nesters, leading to a great lack of knowledge about interspecific competition between more distantly

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related passerines. To determine the existence of interspecific competition between distantly related bird species I selected, for this thesis, three species of insectivorous woodland passerines which are non-hole nesters and belong from three different families. They shift their diet seasonally following the pattern of availability of fruits and insects (Jordano 1982; Herrera 1998; Foster & Robinson 2007; Pejchar 2015) and, they have an intense intraspecific competition which creates a suitable reference for the study of interspecific competition, as their singing and agonistic behaviour is well documented (Cramp & Perrins 1994; Dabelsteen et al. 1997; Matyjasiak 2005).

The first two are European-native species: the European robin *Erithacus rubecula* (hereafter robin; Family Muscicapidae) and the blackcap *Sylvia atricapilla* (Family Sylviidae) are small-sized passerines (weight about 16 g) that are common and widespread across the western Palearctic region (Fig. 1.1; Cramp & Perrins 1994). Their spatial distribution is highly overlapped during the breeding season in the Iberian Peninsula, particularly in Portugal (Martí & del Moral 2004; Equipa-Atlas 2008). The robin and the blackcap share some ecological characteristics since they use the same habitat, have a similar and relatively wide habitat-niche breadth (Seoane & Carrascal 2008), and present similar fruit preferences (Herrera 1998). These ecological similarities have motivated several studies exploring ecological or physiological traits of both species, particularly: their fruit preferences and ecological role in seed dispersal (e.g. Herrera 1998), metabolic activity during the migratory season (e.g. Wirestam et al. 2008), migratory patterns and preferential routes (e.g. Busse et al. 2001), and spatio-temporal distribution (e.g. Tellería & Santos 1993; Pérez-Tris & Tellería 2002b). Considering the ecological and behavioural similarities between the robin and the blackcap, they are likely good candidates to study the putative existence ecological shifting and interspecific competition between distantly related bird species.

To analyse the interactions with a recently-introduced species, I also conducted some experiments with a third species which have a very recent history of coexistence with the robin and the blackcap. The third species is an exotic species without closely related species in Europe: the red-billed leiothrix (hereafter leiothrix; *Leiothrix lutea*, Family Timaliidae; Fig. 1.1) which is small passerine native to south-eastern Asia (weight about 21 g; Male et al. 1998). In the last decades, their popularity as a cage-bird has resulted in several successful introductions in different regions across the world, such as

Hawaii, Japan, and more recently in Europe (Fig. 1.1; Fisher & Baldwin 1947; Amano & Eguchi 2002; Herrando et al. 2010; Farina et al. 2013). Recently, a worldwide-scale meta-analysis on avian introductions considered the leiothrix among the exotic bird species with highest negative impact in bird communities (including competition with native species; Martin-Albarracin et al. 2015). Despite the suggestion that competition exists between the leiothrix and European-native passerines, its competitiveness potential remains unknown (Herrando et al. 2010; Farina et al. 2013; Martin-Albarracin et al. 2015).

Objectives

The main objective of this thesis was to found evidence of competition between these species and, once found, I also traied to understand the ecological and behavioural adaptations that can contribute to reduce it. I used micro-habitat preferences, eco-morphological traits and two behavioural complexes (foraging and singing behaviour) to explore the mechanisms that allow the coexistence of distantly related birds which are potential competitors. The detailed objectives were:

(1) To analyse similarities in the habitat used by robins and blackcaps in coexistence and in isolation aiming to test the existence of habitat shifting between them. If they compete for the breeding habitat, we expect to find differences in its use because of the presence of heterospecifics – **Chapter 2**;

(2) To quantify the levels of aggressiveness in the robin and the blackcap towards conspecifics and heterospecifics, while competing for food, through experimental tests in a controlled environment. If they compete for food, we expect to obtain relatively high frequencies of interspecific aggression – **Chapter 3**;

(3) To identify the potential competitive advantages of the red-billed leiothrix during the establishment process in a community of native passerines by collecting morphological and behavioural data. The exotic species can be considered a better competitor than native species if it has higher exploratory behaviour or more efficient foraging morphology – **Chapter 4**;

(4) To study the possible existence of behavioural dominance of the red-billed leiothrix over native passerines in a feeding context in a controlled environment. We expect that the exotic species should be more aggressive than the native species which should contribute to attain competitive dominance over them – **Chapter 5**;

(5) To test the use of song by the robin and the blackcap as an aggressive signal towards conspecifics and heterospecifics using song playbacks of different species in their natural territories, including native competitors, exotic-competitors and non-competitor species. If robins or blackcaps use the song to signal aggressiveness to heterospecifics, then we expect that: (1) the response towards native competitors should be more aggressive than towards non-competitors; (2) the response towards the exotic competitor should be less aggressive than towards the non-competitor, indicating that they are subordinate to the exotic species – **Chapter 6**.
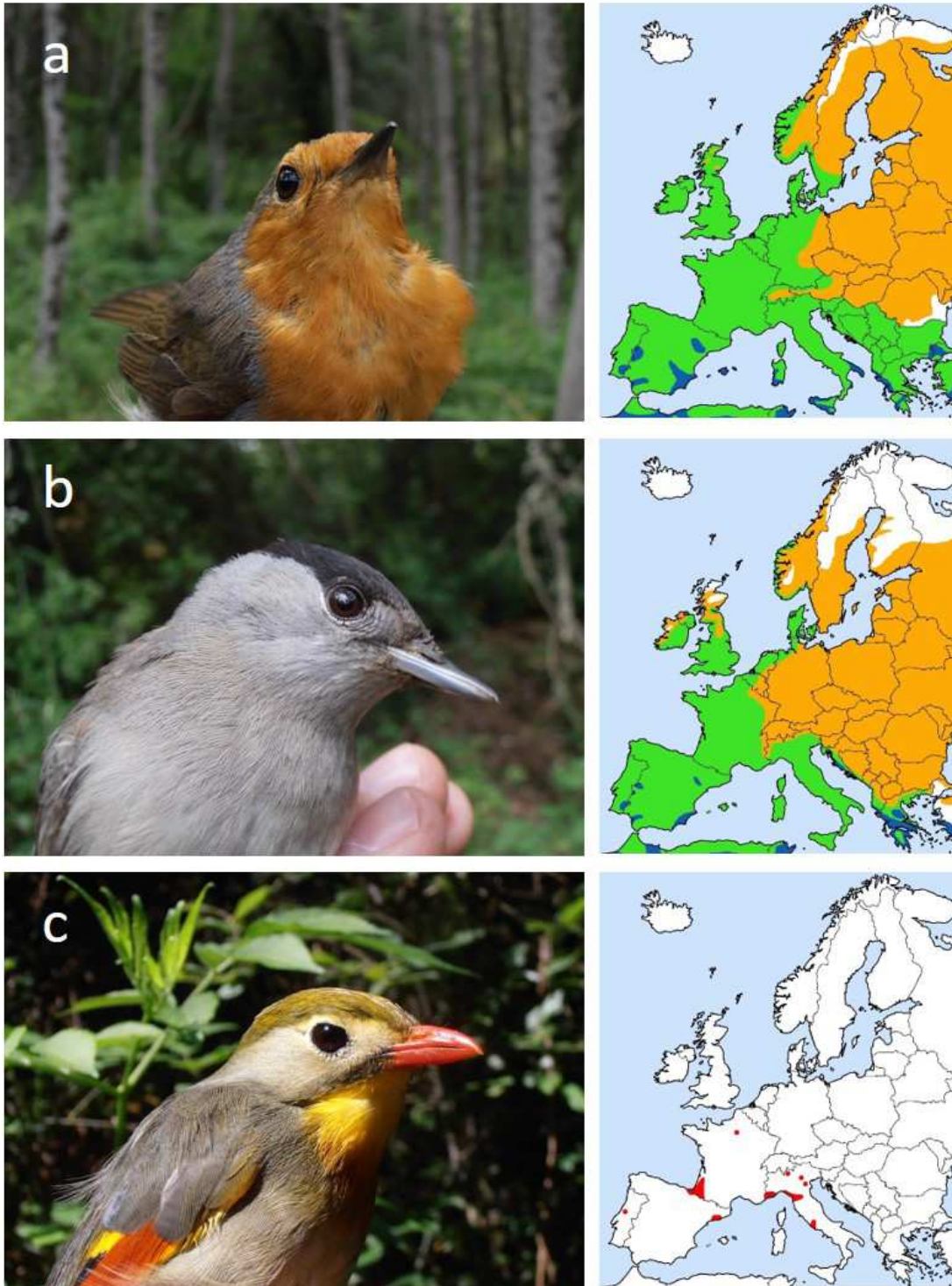
Figure 1.1: Study species and their distribution range in Europe: European robin *Erithacus rubecula* (a), blackcap *Sylvia atricapilla* (b) and red-billed leiothrix *Leiothrix lutea* (c). Colour label: green (resident: present all year), yellow (breeding migrant: present only in spring and summer), dark blue (winter migrant: present only in autumn and winter), red (introduced: present all year). Photo credits: Pedro F. Pereira. Maps adapted from literature (Cramp & Perrins 1994; Martí & del Moral 2004; Equipa-Atlas 2008; Puglisi et al. 2009; Sullivan et al. 2009; Herrando et al. 2011; Farina et al. 2013; Dubois & Cugnasse 2015). Pictures: Pedro F. Pereira

Chapter 2 – Habitat-shift as potential evidence for competition between two distantly related and widespread European Passerines

This chapter was submitted as research article to the "**Ethology Ecology and Evolution**" journal

Authors of the research article:

Pedro Filipe Pereira, Rui Lourenço and Paulo Gama Mota

Abstract

Coexistence between animal species can promote the evolution of mechanisms to avoid or reduce competition, such as ecological shifting. Although taxonomically-distant species can present ecological similarities, ecological shifting has been studied most often between closely-relative species. The European robin *Erithacus rubecula* and the blackcap *Sylvia atricapilla* are two passerines from different families that have a highly-overlapping spatial distribution in the Iberian Peninsula and apparently use the same habitat types. To test the existence of habitat-shift between them, we studied the habitat use at two spatial scales: (1) the landscape scale (broader); and (2) the site scale (fine) where they coexist (syntopy) or only one occurred (allotopy). During the breeding season, we characterized the habitat of each species in Portugal. We measured the importance of each environmental variable with generalized linear mixed models for each species at the two different scales. Both species reached higher abundances in the same landscape type. At the site scale, they used sites with different scrub cover and number of vegetation layers in syntopy while they used the same habitat characteristics in allotopy, suggesting the existence of habitat-shift. Our study indicates that the two distantly related passerines have similar habitat use at the landscape scale and present some degree of habitat-shift. These are evidences that may play an important role in community structure even between birds from different families.

Keywords: allotopy, ecological shift, *Erithacus rubecula*, habitat use, *Sylvia atricapilla*, resource partitioning, syntopy

Introduction

Potentially direct competitors may overlap extensively in their geographical distributions (e.g. Beaver & Baldwin 1975; Robinson 1981; Minot & Perrins 1986; Davies et al. 2007; Lisičić et al. 2012). However, the mechanisms allowing for the coexistence of competitors may vary according to the spatial scale of analysis (Wiens 1989; Huston 1999; Kneitel & Chase 2004). For example, the ability to compete for limiting resources shapes the coexistence of species only at a relatively small spatial scale (Huston 1999; Kneitel & Chase 2004). In general, species with wide geographical distributions or broad niches are more prone to develop ecological shifting with direct competitors (i.e. change some of their ecological characteristics as a consequence of a competitive pressure; Connell 1980; Case et al. 2005; Davies et al. 2007). Some studies that compare the habitat used by closely-related species in sympatric and allopatric conditions found evidences of different use in sympatry (i.e. resource partitioning) or similar use in allopatry evidencing the existence of ecological shifting between them (Rivas 1964; Beaver & Baldwin 1975; Jenssen et al. 1984; Lisičić et al. 2012).

Many sympatric bird species use relatively similar ecological niches along extensive overlapping distributions, which can potentially lead to strong direct competition. Among terrestrial vertebrates, passerine birds are one of the most well studied groups in terms of ecological shifts (e.g. Beaver & Baldwin 1975; Robinson 1981; Minot & Perrins 1986; Dayan & Simberloff 2005; Grether et al. 2009). However, most of these studies were focused on competition between closely-related species, whereas competition involving taxonomically distant species remains poorly studied (Dayan & Simberloff 2005).

The European robin *Erithacus rubecula* (henceforth robin; Family Muscicapidae) and the blackcap *Sylvia atricapilla* (Family Sylviidae) are small woodland passerines that

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are common and widespread across the western Palearctic region (Cramp & Perrins 1994). During the breeding season, they are territorial and compete with conspecifics for mates and nesting sites (e.g. Tobias & Seddon 2000; Leedale et al. 2015). Such as several territorial passerine birds, robins and blackcaps use the song to defend the territory where they develop all activities, including foraging and nesting (Brown 1964; Cramp & Perrins 1994). Some authors suggested the existence of great differences between the two species in their tolerance to environmental seasonality, referring that the robin is less tolerant to drought and more tolerant to winter severity than the blackcap (Pérez-Tris & Tellería 2002a; Tellería 2015). Accordingly, Pérez-Tris & Tellería (2002a) correlated the environmental seasonality with the migratory behaviour of both species and found that blackcaps show a stronger migratory behaviour than robins. Considering these studies, the ecological differences reduce the coexistence, thus suggesting the inexistence of interspecific competition between the two species. However, in Portugal, the breeding populations of both species are resident (Catry et al. 2010; Campos et al. 2011b; Andrade et al. 2015). Moreover, robins and blackcaps show similar use of space at different scales which make them good candidates to test the existence of habitat-shift between unrelated bird species because: (1) at regional scale, their breeding distribution range highly overlaps in the Iberian Peninsula and particularly in Portugal (Fig. 2.1; Martí & del Moral 2004; Equipa-Atlas 2008); also the population densities of both species appear to increase as the rain fall increase across the Iberia (Tellería & Santos 1993); (2) at the landscape scale, they appear to use the same habitat types and show similar and wide niche breadth (Blondel & Farré 1988; Farina 1995; Preiss et al. 1997; Seoane & Carrascal 2008); (3) at site scale, they select places with similar vegetation characteristics, such as trees or arborescent scrubs (which creates a net of suitable singing perches), several vegetation layers and dense

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undergrowth (which generates suitable conditions for their nesting; Cramp & Perrins 1994; Carbonell & Tellería 1998; Laiolo 2002; Martí & del Moral 2004; Bracken & Bolger 2006; Camprodon & Brotons 2006); (4) interspecific competition has been recorded between the two species, including the use of aggressive behaviour (Leach 1981; Hernández 2008).

To test for the possible existence of habitat-shift between robins and blackcaps, we studied habitat use by each species at two different scales: the site scale (fine) and the landscape scale (broad). These two scales are relatively close to the individual scale, which is the level that species interact directly (Huston 1999), and thus can be considered more suitable to evaluate the existence of habitat shifting than a broader scale (e.g. regional scale). Accordingly, if the robin and the blackcap show habitat-shift during the breeding season, we would expect to find (1) similar habitat use at a landscape scale, (2) different habitat use at a site scale where species are in sympatric conditions, indicating resource-partitioning and (3) similar habitat use at a site scale when species are in allopatric conditions. On the contrary, if these species show no habitat-shift, we would expect to find different habitat use between both species in sympatric as well as in allopatric conditions.

Methods

Study area

In Portugal, the robin and the blackcap occur mostly in sympatry (Fig. 2.1; Equipa-Atlas 2008). Therefore, we selected six study areas across the country (area 1 to area 6; Fig. 2.1) with different occurrence frequencies of each species aiming to find sympatric and allopatric conditions at a landscape scale, i.e. syntopy and allotopy, respectively

(Rivas 1964). The average distance between adjacent areas was 133 ± 25 km (mean ± standard deviation). The set of areas covers different climate types (temperate or Mediterranean) and roughly the entire latitudinal range of both species distribution in Portugal (Appendix 2.1). Within each area, we selected three habitats which are the main wood-types across the Portuguese territory: (1) exotic woods, (2) pinewoods and (3) native broadleaf woods (deciduous in areas 1 and 3; deciduous-evergreen mixed woods in the remaining areas; Appendix I). This wood-types can be arrayed according to their increasing naturalness degree from 1 to 3 (Proença et al. 2010). Exotic woods are dominated by Australian trees (cultivated *Eucalyptus* spp., plus invasive *Acacia* spp. in some areas) which were novel habitats for both study species, after their introduction in the country in mid. 20th century. Pine-woods present an intermediate degree of naturalization, since they were cultivations of European native species (*Pinus pinaster*, *P. pinea*, *P. nigra*).

Data collection and habitat characterization

Each area was visited once between mid-April and late May 2013, during the breeding season of robins and blackcaps. Since these species are detected mainly by their song, we followed a south – north order to reduce the differences in the frequency of singing behaviour between areas. We conducted 30 linear transects in each study area. We recorded every territorial singing males of each species (termed here as abundance) within the area of the transect (50 m wide to each side of the transect x 300 m length) and plotted them into a map (Appendix I). We spent at least 30 minutes in each transect considering it as a suitable period to record all individuals within its area (100 m x 300 m). We characterized the habitat at two different spatial scales: the landscape scale (the area within the transect) and the site scale (the area within a circle of 10 m radius). The area covered by a site should comprises about ¼ of the average territory size of a bird

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(mean ± standard deviation: 1387 ± 621 m² for robins (n = 16 territories) and 1183 ± 636 m² for blackcaps (n = 16 territories); PFP unpublished data). We characterized two types of sites to measure the habitat availability (details in the data analysis section): occupied-sites (with singing robins or blackcaps) which indicated the habitat use of each species and empty-sites (which were randomly selected sites without the target species). To avoid "false" empty-sites, the minimum distance between the central points of two adjacent sites was 50 m.
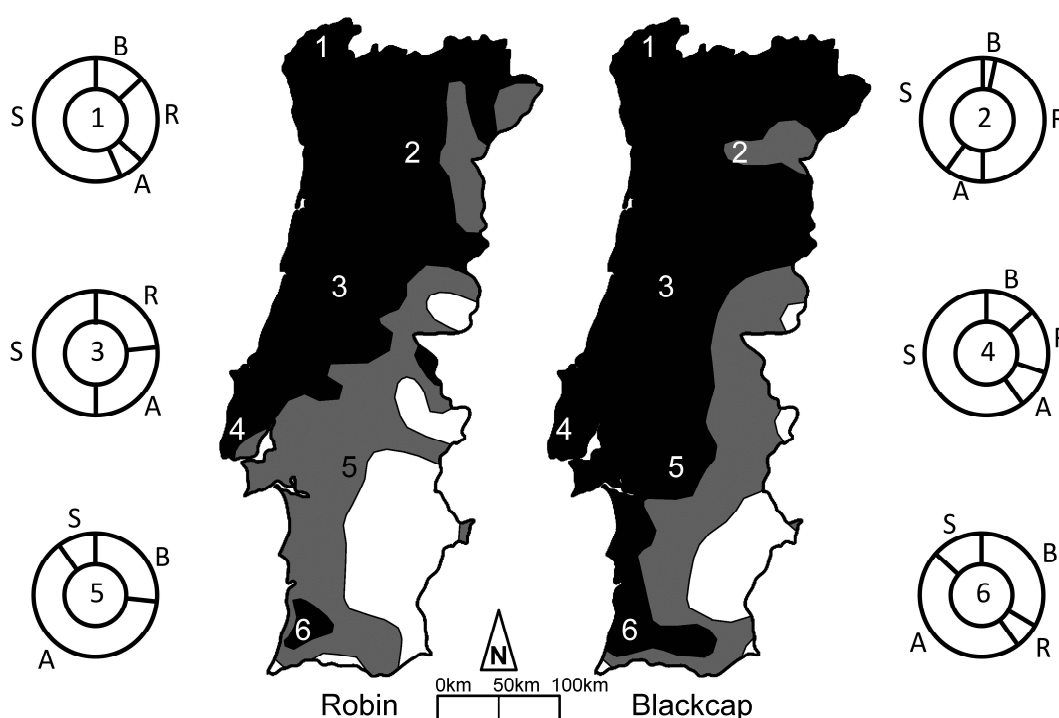
Fig. 2.1: Occurrence frequency of robin and blackcap in Portugal during the breeding season, showing the location of the six study areas (1 – 6). Maps show three classes of frequency: more frequent (black), less frequent (grey), very rare or absent (white). Circular plots show the proportion of transects sampled in this study where robins and blackcaps were syntopic or allotopic (sample size per area = 30 transects): syntopy (S), allotopic robins (R), allotopic blackcaps (B), absence of both species (A). Maps are in accordance with the Portuguese Breeding Bird Atlas (adapted from Equipa-Atlas 2008).

In each area, we conducted 10 transects per each class of wood-type (exotic, pine or native broadleaf woods). We classified the matrix-type as the type of habitat 50 m around the transect area as (1) farmland (n = 28), (2) mixed farmland-woodland (n = 75) or (3) woodland (n = 77). The sun exposure of transects was ranked in four classes: (1) sunny, for transects on hillsides facing SW, S, SE or E, which are hotter and drier than average climate conditions in each area (n = 28); (2) average, which included both low land plains and plateaus (n = 93); (3) shady, for transects on hillsides facing W, NW, N and NE (n = 38); or (4) moist for transects along close stream valleys which tend to be more temperate than the average micro-climate of the area (n = 21). Altitude was measured at the central point of each transect (mean ± standard deviation: 331.8 ± 227.3 m a.s.l.; range: 25.5 – 964.0 m a.s.l.).

Per transect, we sampled a maximum of three occupied-sites by each species and a similar number of empty-sites. In both types of sites (occupied or empty), we estimated the ground area covered by the horizontal projection of tree crowns (≥ 2 m height and single-trunked plants) and/or scrubs (< 2 m height or multiple-branching plants) and the number of vegetation layers as the sum of the number of trees and scrubs differing in their height. Vegetation height was measured for scrubs and for the tallest woody-plant in the site (maximum height). This habitat characterization was based on a priori knowledge of the most relevant habitat characteristics for both species. When a singing male moved between different perches, we characterized the occupied-site where the bird sang first. Adjacent singing males (perched closer than 10 m) were excluded from the site scale study, since these individuals could be near their territory borders, therefore biasing characterization.

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Statistical Analysis

Data was analysed using a multimodel inference approach to cope with model selection uncertainty (Burnham & Anderson 2002). All variables were analysed for distribution and to detect outliers, with all being normally distributed. All variable combinations had low collinearity ($r_P < 0.7$), and thus were included in the following analyses.

We performed two analyses at landscape scale using generalized linear mixed models with Poisson distribution and considering the six study areas as a random factor: *robin landscape* and *blackcap landscape* analyses, which used, respectively, robin and blackcap abundances (number of singing males) as response count variable. Wood-type, matrix-type, sun exposure and altitude were used as explanatory variables. Both analyses included all sampled data (n = 180 transects), with the main goal being to characterize the habitat use of each species at a landscape scale.

Two analyses at site scale were carried out using generalized linear mixed models with a binomial distribution and considering the transect ID of each occupied site as a random factor: one using sites located in transects with both species (*syntopy analysis*) and another using sites located in transects with just one of the target species (*allotopy analysis*). Analyses at site scale allow the detection of environmental traits which are important for one species but not for the other, i.e. the resource partitioning between species. We expected to find differences between the habitat use of the robin and the blackcap in syntopy but not in allotopy, i.e. the existence of habitat-shift between these species. We measured how much the available habitat was used for each environmental variable (number of vegetation layers, maximum height of vegetation, tree cover, scrub cover and scrub height) by: (1) determining the maximum value for each environmental variable using all sites of a transect; and (2) calculating the relative percentage of each variable for the occupied-sites in proportion to their maximum value in the respective

transect (Table 2.1). As an example, if the maximum number of vegetation layers recorded in each transect was 4 and the number of layers in an occupied-site was 3, the relative percentage was 75%. The relative percentage of each habitat characteristic was used as independent variables. In the *syntopy analysis*, we included 196 sites (93 occupied by robins and 103 occupied by blackcaps) and in the *allotopy analysis* we included 64 sites (33 occupied by robins and 31 by blackcaps). Additionally, we sampled 155 empty-sites in syntopy and 121 in allotopy. We used a binomial response variable (robin/blackcap) in both site scale analyses.

All competing models used in both landscape- and site scale analyses were built with a maximum of three variables to avoid over-fitting. We generated all possible model combinations, since we consider that all explanatory variables were a priori important to the response variables, and because we were interested in their effect and order of importance. The null model was included in each multimodel inference analysis as an indicator of model performance. Models were ranked according to their AIC_c values. The AIC_c difference (ΔAIC_c) and the Akaike weight (w_i) were also determined (Burnham & Anderson 2002), and the set of best models were those with $\Delta AIC_c < 2.00$ (Burnham & Anderson 2002). Model-averaged coefficients of all explanatory variables were also determined. Finally, the relative variable importance (RVI) of each explanatory variable was calculated as the sum of the w_i of all the models in which the variable was included (Burnham & Anderson 2002). Model results were validated using diagnostic plots. All analyses were performed using the software R 3.3.0 (R-Core-Team 2016) with the packages gplots (Warnes et al. 2013), nlme (Pinheiro et al. 2007) and MuMIn (Barton 2013).

Table 2.1: Explanatory variables used in site scale analyses, showing its mean ± standard deviation (range). Values are presented in percentage relating the use of each species with the range of each habitat characteristic available in the transect. See methods for details.

Variable	Description	Syntopy	Allotopy
Vegetation layers	Number of woody vegetation layers	79.5 ± 20.4 (20 - 100)	80.2 ± 22.9 (25.0 - 100)
Maximum height	Maximum height of woody vegetation	77.1 ± 25.7 (8.9 - 100)	82.9 ± 19.8 (22.7 - 100)
Tree cover	Percentage of ground covered with the horizontal projection of tree canopy	59.6 ± 38.4 (0 - 100)	76.4 ± 34.8 (0 - 100)
Scrub cover	Percentage of ground covered with scrub	70.5 ± 29.0 (0 - 100)	66.3 ± 29.3 (0 - 100)
Scrub height	Maximum height reached by scrub	64.5 ± 29.3 (0 - 100)	70.5 ± 30.4 (0 - 100)

Results

A total of 254 singing robins and 231 singing blackcaps were recorded in this study. The target bird species were recorded in ca. 73% of the total of 180 transects: 69 transects with syntopic conditions distributed by all study areas, 35 transects with allotopic robins (in all areas, except in area 5) and 27 transects with allotopic blackcaps (in all areas, except in area 3; Fig. 2.1).

Landscape scale habitat

We obtained a single best model for the *robin landscape* analysis, as well as for the *blackcap landscape* analysis (Table 2.2; Appendix 2.2). Wood-type was included in the best models of each analysis and showed a very high relative variable importance for the *robin landscape* (RVI = 0.99) and for the *blackcap landscape* (RVI = 1.00). The robin and the blackcap had higher abundances in native broadleaf woods than in other wood-types (Fig. 2.2.a), which agreed with our prediction that both species have similar habitat use at a landscape scale.

Site scale habitat

Robins and blackcaps used sites where all habitat characteristics were on average above 50% of their availability within the transect (i.e. denser, taller and with more vegetation layers than the average sites; Fig. 2.3). In the *syntopy analysis*, we obtained five best models, which included all variables (Table 2.3; Appendix 2.3). The number of vegetation layers (RVI = 0.71) and scrub cover (RVI = 0.68) showed greater relative variable importance, thus being the main factors separating the sites that each species uses. Sites used by robins apparently had more vegetation layers and lesser scrub cover than sites used by blackcaps (Fig. 2.3.a and 2.3.e).

In the *allotopy analysis* the null model was included in the set of best models, meaning that both species overlap considerably for every trait variable measured. It is thus reasonable to assume that there are no strong differences in the habitat used by both species in areas where only one species occurs (Table 2.3; Fig. 2.3; Appendix 2.3). These results agree with our prediction of similar habitat use at a site scale where species occur in allotopic conditions.

Table 2.2: Relative variable importance (RVI) in the landscape scale analyses (multimodel inference) analysing the effect of landscape characteristics on the abundance of robins and blackcaps (*robin landscape* and *blackcap landscape*, respectively). The asterisk indicates the variables included in the best models (ΔAIC c < 2.00).

	RVI	
Variables	**Robin landscape**	**Blackcap landscape**
Wood-type	0.99*	1.00*
Matrix-type	0.08	0.07
Sun exposure	0.03	0.17
Altitude	0.02	0.00
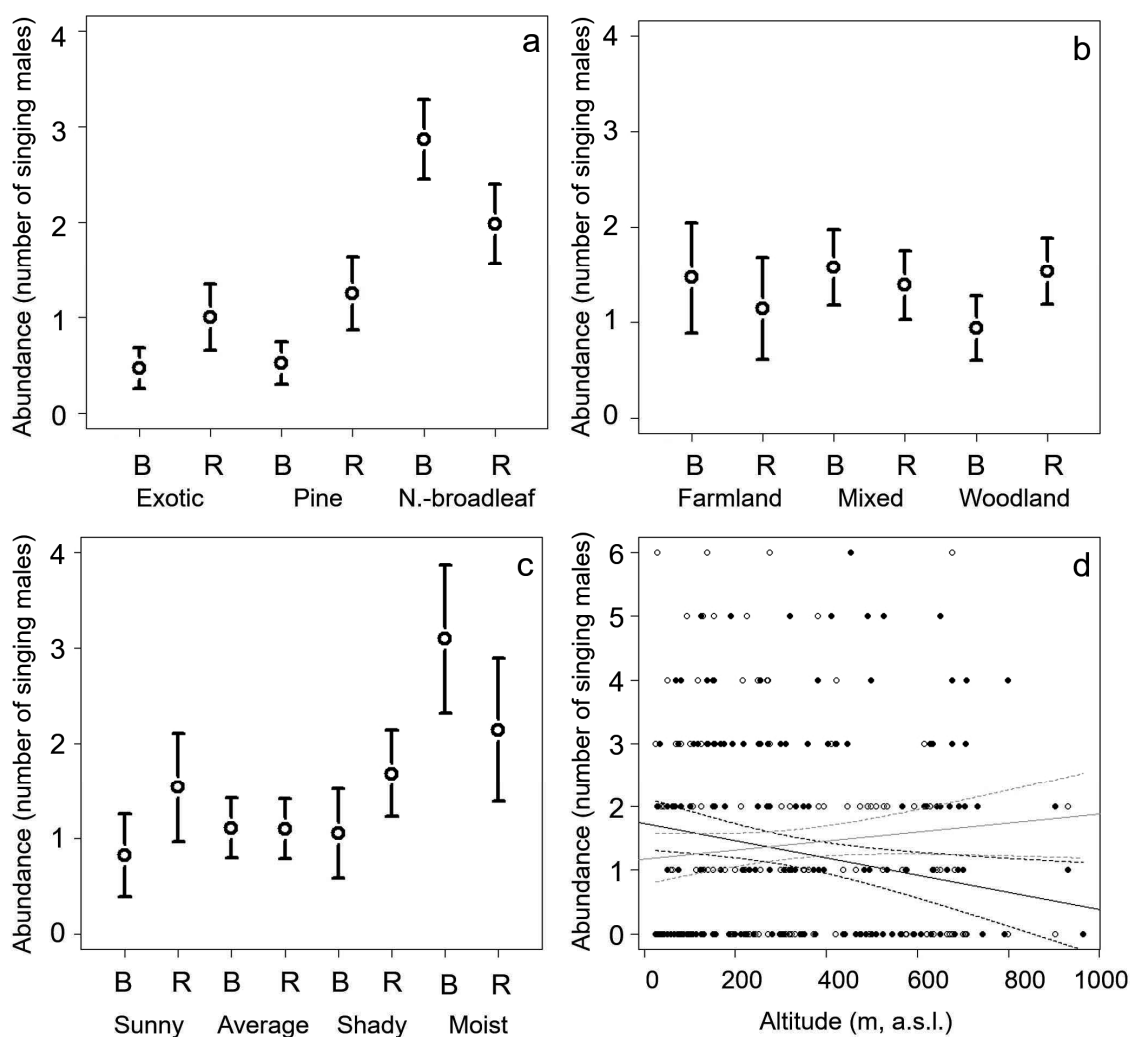
Fig. 2.2: Mean abundance (number of singing males) of blackcaps (B) and robins (R) according to four landscape scale variables: wood-type (a), matrix-type (b), sun exposure (c) and altitude (d). Confidence intervals of 95% are represented by bars in figures a-c and by lines in figure d. In figure d, black and white dots represent blackcap and robin abundances, respectively.

Table 2.3: Relative variable importance (RVI) in the site scale analyses (multimodel inference) testing the effect of site characteristics in transects where robins and blackcaps coexist and in transects where they did not (*syntopy* and *allotopy*, respectively). The asterisk indicates the variables included in the best models (ΔAICc < 2.00).

Variables	RVI	
	Syntopy	Allotopy
Vegetation layers	0.71*	0.24
Maximum height	0.20*	0.23
Tree cover	0.27*	0.26
Scrub cover	0.68*	0.35
Scrub height	0.31*	0.25
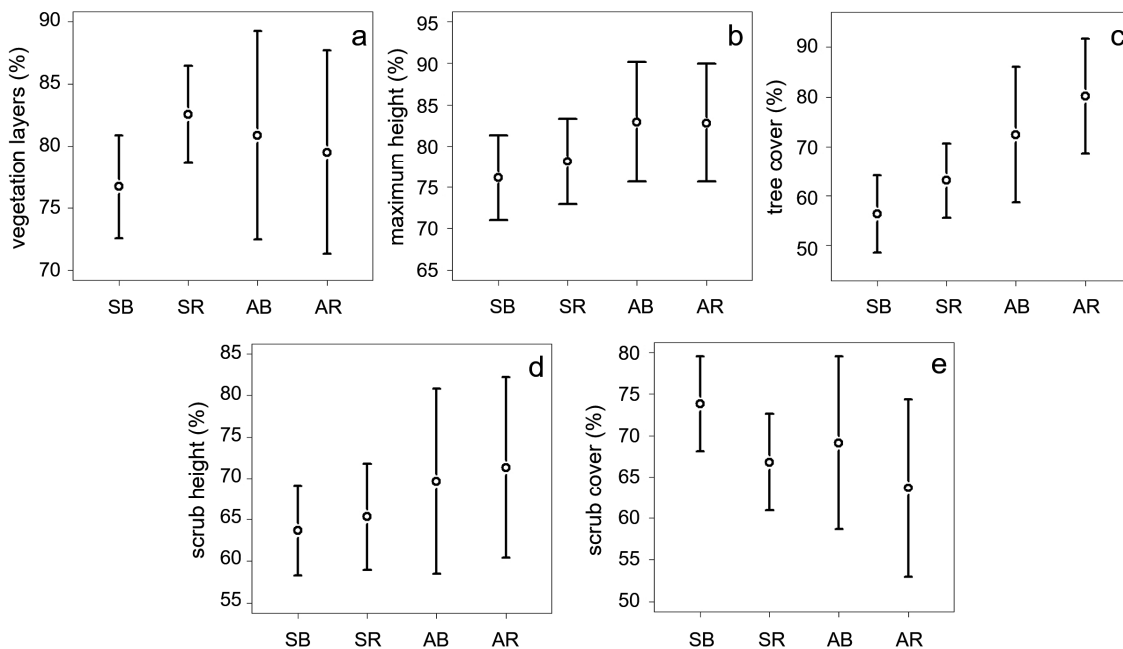
Fig. 2.3: Use of habitat characteristics at site scale by robins and blackcaps in transects in syntopy and allotopy: syntopic blackcaps (SB), syntopic robins (SR), allotopic blackcaps (AB) and allotopic robins (AR). Values are presented in percentage relating the use of each species with the range of each habitat characteristic available in the transect. Confidence intervals of 95% are represented by bars. See methods for details.

Discussion

Landscape scale habitat

At a landscape scale, the robin and the blackcap were influenced by the same characteristics, since both species were more abundant in native broadleaf woods than in other type of woods. Our results also indicate that both species were much less affected by altitude, sun exposure and matrix-type than by the wood-type. A lesser use of conifer or exotic plantations by these species was also noted in other regions (Russia: Greenberg et al. 1999; Spain: Martí & del Moral 2004; Azores: Ceia et al. 2009; Greece: Kati et al. 2009; Britain: Hewson et al. 2011). When compared with pine or exotic plantations, native broadleaf woods are richer in food resources, nesting or refuge sites, incorporating a greater variety of plants and invertebrates (and having a greater diversity of specialized species), which are generally more sensitive to human management (Finch 2005; Wiezik et al. 2007; Proença et al. 2010; De la Hera et al. 2013). Moreover, native broadleaf woods have more naturalness than plantations as their life-cycles are synchronized with the remaining system, particularly with the reproductive activity of birds (Remeš 2003; De la Hera et al. 2013). As an example, exotic woods with an early timing of leafing can be "ecological traps" for birds since they allow lower nesting success than native woods (Remeš 2003). Our results show that robins and blackcaps can coexist at a smaller scale than the peninsular or country scales. The coexistence of species at a relatively small scale (such as the landscape scale) allows the interaction between individuals being the level in which the species compete directly (Huston 1999).

Site scale habitat

The high importance (RVI) of vegetation layers and scrub cover in the *syntopy analysis* suggests that robins and blackcaps differed in habitat when they coexist, i.e. the existence of resource partitioning between the two species. Sites used by robins had higher number of vegetation layers (i.e. greater vertical stratification) than those used by blackcaps, whereas the latter species used sites with denser scrub cover. Vertical stratification of woody vegetation reduces insolation and drought of soil, which can be particularly important to reduce the severity of Mediterranean summers (Sánchez et al. 2010). Moist soil increases the abundance of invertebrates which is critical for the breeding success of ground foragers, such as the robin (Ludvig et al. 1995; Tellería 2015). During the breeding season, robins present greater variation in abundances between climate regions than blackcaps (Pérez-Tris & Tellería 2002a), which suggests that robins are more dependent on suitable micro-climatic conditions that are created by the presence of several vegetation layers. Beyond that, sites with dense scrub cover are likely more important for blackcaps than for robins, since blackcaps have more strict nesting substrate preferences than robins (Cramp & Perrins 1994; Camprodon & Brotons 2006). Also, robins can benefit with moderate scrub clearing in densely covered areas and the consequent increase of the ground-surface available for foraging (Fennessy & Kelly 2006; Tellería 2015). However, the evidence of resource partitioning between species is not enough to exclude the prevalence of interspecific competition, although it should contribute to reduce the strength of this interaction. That is the case with the garden warbler *Sylvia borin*, which has a geographically restricted distribution in Iberian Peninsula, and prefers sites with lower vegetation height and sparse tree cover when compared to the blackcap in Northern Europe (Cody 1978; Garcia 1983; Tellería & Santos 1993); however, strong interspecific competition between the blackcap and

the garden warbler was experimentally demonstrated (Cody 1978; Garcia 1983; Matyjasiak 2005). In summary, our results indicate that resource partitioning in habitat use exists between robins and blackcaps at a site scale when species occur in syntopy. On the contrary, when considering the allotopic conditions, there were no differences in habitat used by both species. Thus, when isolated from each other, the robin and the blackcap used sites with similar vegetation characteristics, including similar number of vegetation layers and scrub cover. Considering that they tend to segregate in syntopy while use the same habitat in allotopy, our results suggest the existence of habitat-shift between these species.

Our study shows that two distantly related passerine birds, but with similar distribution ranges in Iberian and country scales, can also have similar habitat use at a landscape scale. Moreover, at the site scale – the scale in which the individuals interact directly and compete for resources (Wiens 1989; Huston 1999; Kneitel & Chase 2004) – the habitat used by the robin and the blackcap was affected by the coexistence with the other species, which is an indicator of the existence of interspecific competition. Interestingly, a recent work (Losin et al. 2016), focusing in a large group of closely related passerines with similar ecologies, found that the syntopy between species was an important predictor of interspecific territoriality. Therefore, studies between distantly related species that show habitat shifting (such as the robin and the blackcap) should be conducted to evaluate the effect of syntopy in interspecific territoriality. The existence of a habitat-shift between distantly related species may have a role in community structure which in general has been seldom taken in consideration, and thus should receive more attention from researchers in ecology and evolution.

Appendix 2.1

Geographical and bio-climatic description of study areas: area (code – name; central coordinates), altitude range (m a.s.l.), average annual temperature (ºC), average rain fall (mm). For each wood-type (exotic, pine and native broadleaf woods) the species or genus presented in all replicates were listed.

Area	Altitude range	Average annual temperature; average rain fall	Wood-type Exotic	Pine	Native broadleaf
1 – Minho; 41°52'N and 8°33'W	50-800	13; 2000	*Eucalyptus* spp.	*Pinus pinaster*	*Quercus robur*
2 – Douro; 41°09'N and 7°33'W	100-800	14; 800	*Eucalyptus globulus*	*Pinus pinaster*	*Quercus suber* and *Castanea sativa*
3 – Coimbra; 40°02'N and 8°16'W	140-1,200	12; 1500	*Eucalyptus gobulus* and *Acacia* spp.	*Pinus* spp.	*Quercus robur* and *Castanea sativa*
4 – Sintra; 38°49'N and 9°24'W	0-400	14; 800	*Eucalyptus globulus* and *Acacia* spp.	*Pinus pinea*	*Fraxinus angustifolia* and *Olea europaea* var. *sylvestris*
5 – Évora; 38°32'N and 8°18'W	50-400	16; 700	*Eucalyptus globulus*	*Pinus pinea*	*Fraxinus angustifolia* and *Quercus suber*
6 – Monchique; 37°18'N and 8°42'W	20-900	15; 1000	*Eucalyptus* spp.	*Pinus pinaster*	*Alnus glutinosa* and *Quercus suber*

Appendix 2.2

Statistical parameters of the best models (ΔAIC c < 2.00) from the landscape scale analyses (multimodel inference) testing the effect of landscape characteristics on the abundance of robins and blackcaps (*robin landscape* and *blackcap landscape*, respectively). Variable code: wood type (1).

Analyses	Combination of variables	df	logLik	AICc	ΔAICc	Weight
Robin landscape	1	5	-301.65	613.65	0.00	0.87
Blackcap landscape	1	5	-277.47	565.29	0.00	0.78

Appendix 2.3

Statistical parameters of the best models (ΔAIC c < 2.00) from the site scale analyses (multimodel inference) testing the effect of site characteristics in the presence of robins and blackcaps when they occur in *syntopy* and *allotopy*. Variable codes: maximum height (1), scrub height (2), scrub cover (3), tree cover (4), vegetation layers (5).

Analyses	Combination of variables	df	logLik	AICc	ΔAICc	Weight
Syntopy	35	4	-131.62	271.45	0.00	0.20
	235	5	-130.93	272.17	0.72	0.14
	345	5	-131.42	273.16	1.71	0.08
	5	3	-133.55	273.23	1.78	0.08
	135	5	-131.55	273.41	1.96	0.07
Allotopy	Null Model	2	-36.54	77.28	0.00	0.19

Chapter 3 – Heterospecific aggression over feeding by two sympatric birds differing in territorial behaviour

This chapter was submitted as research article to the "**Ethology**" journal

Authors of the research article:

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Abstract

Interspecific competition affects the behaviour of individuals, and ultimately influence population dynamics. Despite its relevance on species evolution, interspecific competition has been less studied compared to intraspecific competition. Most studies exclude the effect of territorial status of species, although it may affect the use of aggressiveness when disputing resources. During the non-breeding season, we designed an experiment aiming to (1) test the existence of heterospecific aggression and (2) measure its significance in relation to the conspecific aggression over food and space between two phylogenetically-distant species that have similar feeding preferences: the territorial robin *Erithacus rubecula* and the non-territorial blackcap *Sylvia atricapilla*. We used pairs of opponents in heterospecific and conspecific experiments. Considering the differences in territorial behaviour between the two species, we predicted that (1) in conspecific interactions, robins should be more aggressive than blackcaps; (2) conspecific aggressiveness between robins should be more intense than heterospecific aggressiveness; (3) most social interactions involving blackcaps should be non-aggressive. For both species, the latency to approach did not differ between experiments, suggesting heterospecific-rival recognition. Robins attacked conspecifics more frequently than heterospecifics, while blackcaps presented lower but similar frequencies of aggressive behaviours between conspecific and heterospecific experiments. The aggressive responses of each species against conspecifics and heterospecifics can be related to variations in costs and benefits resulting from their differences in territorial behaviour. We concluded that it is worth investigating heterospecific aggression even in phylogenetically-distant species, as it may be a relevant indicator of interspecific competition.

Keywords: Blackcap, *Erithacus rubecula*, interspecific aggressiveness, competition, European robin, *Sylvia atricapilla*

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Introduction

Competition for resources may occur both within and among species. Interspecific competition can shape the behaviour of individuals, and ultimately affect population dynamics through niche occupancy (Mac Nally 1983; Schoener 1983; Pigot & Tobias 2013). Despite the relevance of interspecific competition on the evolution of species and structuring of communities, it has been less studied compared to intraspecific competition (Grether et al. 2009; Peiman & Robinson 2010; Grether et al. 2013). Interspecific competition can result from exploitation, when the resources used by one species reduce their availability for competing species (Minot 1981; Petren & Case 1996), or interference, when one species restricts the access to resources to a competing species (Schoener 1983; Walls 1990; Downes & Bauwens 2002; Eccard et al. 2011). Aggressive interference mechanisms may include chemical competition, aggressive behaviour and, in their extreme, killing the competitor (Lourenço et al. 2014).

Aggressive interactions between organisms frequently result from competition for limited resources (e.g. food, mates or nest sites; Ostfeld 1985; Gabor & Jaeger 1995; Duckworth 2006). Generally, the willingness to dispute resources depends on a trade-off between the costs and benefits for individuals in obtaining them (Smith 1982). When resources are susceptible to being monopolized, individuals may defend territories for exclusive access, reducing the costs of disputes (Maher & Lott 1995). When territories are not defensible, mechanisms allowing for decisions over disputes with a minimum risk of injury are expected to evolve, such as the establishment of dominance ranks and status signalling (Lange & Leimar 2003; Pryke & Andersson 2003; Peiman & Robinson 2010). Consequently, non-territorial individuals in competition contexts tend to avoid aggressive contests when interacting with each other,

as well as with territorial individuals (Pryke & Andersson 2003; Gauvin & Giraldeau 2004).

Interspecific aggression (heterospecific aggression synonym) has a high prevalence in nature, including among invertebrates, fishes or terrestrial vertebrates (e.g. Orians & Willson 1964; Menge & Menge 1974; Walls 1990; Duckworth 2006; Forrester et al. 2006; Eccard et al. 2011). In general, the intensity of aggressive behaviour towards conspecifics and heterospecifics is expected to differ (Walls 1990; Martin et al. 1996; Downes & Bauwens 2002; Forrester et al. 2006; Freeman 2016). Aggressiveness should be more frequent between conspecifics than between heterospecifics, because individuals are expected to be less willing to approach and interact with heterospecifics than conspecifics, due to a greater uncertainty of interaction outcomes, with increased risk of injuries and fighting costs (Peiman & Robinson 2010). However, the effect of territoriality in the heterospecific aggressiveness of individuals it is not clear (Greenberg 1986; Grether et al. 2013). Accordingly, some authors found that both territorial and non-territorial species were more aggressive towards conspecifics than towards heterospecifics (hummingbirds: Powers & Conley 1994; parrotfishes: Muñoz & Motta 2000). In turn, Garcia and Arroyo (2002), in a study with birds of prey, found that the non-territorial species was more aggressive towards conspecifics while the territorial species was similarly aggressive towards both conspecifics and heterospecifics. However, these studies were conducted during the breeding season when competition for mating should have an important contribute to intraspecific competition (Peiman & Robinson 2010; Grether et al. 2013). Therefore, to exclude the effect of mate competition, it is necessary to quantify the significance of heterospecific aggressiveness

in relation to the conspecific aggressiveness during the non-breeding season, particularly if species differ in territorial habits.

We studied two small passerine species with similar body-size, the European robin *Erithacus rubecula* (henceforth robin; family Muscicapidae) and the blackcap *Sylvia atricapilla* (family Sylviidae), which are abundant in the Western Palearctic, particularly in Mediterranean countries (Cramp & Perrins 1994; Herrera 1998). They have a different social organization since robins are territorial throughout the year, while blackcaps are territorial during the breeding season and show a wandering behaviour in the remaining part of the year (Schwabl 1992; Cuadrado 1997; Pérez-Tris & Tellería 2002a; Tellería & Pérez-Tris 2003; Hernández 2008; Leedale et al. 2015). In spite of this, we opted to study the two species outside the breeding season in order to avoid the effect of reproductive competition between conspecifics. The robin and the blackcap are potential candidates for the occurrence of heterospecific aggression outside the breeding season because: (1) they present strong intraspecific competition for feeding resources (Leach 1981; Cuadrado 1997; Pérez-Tris & Tellería 2002b; Tellería & Pérez-Tris 2003; Catry et al. 2004; Campos et al. 2011a; Morganti et al. 2017); (2) they are abundant in the same habitats, such as scrublands, olive groves and woodland habitats (Pérez-Tris & Tellería 2002a); (3) they feed on insects and fruits which are limiting resources during the winter (Cramp & Perrins 1994; Pérez-Tris & Tellería 2002a; Hernández 2008; Campos et al. 2011a); (4) they are the main frugivorous species within their distribution ranges and have similar fruit preferences (Jordano 1989; Herrera et al. 1994; Herrera 1998); (5) they use similar foraging techniques (mostly gleaning; Jordano 1982). Despite their potential as heterospecific competitors, aggressive interactions between them are not often observed in nature, because it

requires very long observation times (Orians & Willson 1964; Jordano 1982; Cuadrado 1997; Hernández 2008; Morganti et al. 2017). Consequently, only a few studies recorded instances of heterospecific aggression between the two species (Leach 1981; Hernández 2008). Thus, we designed a field experiment of competition within and between robins and blackcaps over food and space in an enclosed artificial environment opposing pairs of conspecifics and heterospecifics. Our objectives were: (1) to determine if two passerine species differing in their territorial habits also differ in the intensity of their heterospecific aggression; and (2) to measure the significance of heterospecific aggression in relation to the conspecific aggression in both species. We also recorded non-aggressive behaviours in order to account for their significance among all social interactions. Considering the differences in social organization between the two species, we predicted that (1) in conspecific interactions, the territorial species – the robin – should be more aggressive than the non-territorial species – the blackcap, since territoriality is an outcome of conspecific aggressiveness (Maher & Lott 1995); (2) conspecific aggressiveness in robins should be more intense than heterospecific aggressiveness, due to a greater certainty about fighting costs among conspecifics (Peiman & Robinson 2010); (3) most social interactions involving blackcaps should be non-aggressive, since non-territorial individuals tend to avoid aggressive contests (Pryke & Andersson 2003; Gauvin & Giraldeau 2004).

Methods

Study area

The study was performed in central Portugal (40° 3.162'N, 8° 20.540'W) in a deciduous oak-woodland (*Quercus robur*).

Captures and field enclosure experiment

To minimize observer bias, blinded methods were used while recording and analysing all behavioural data. Field work was performed between early November 2014 and late February 2015. Birds were captured between 7:00 am and 3:30 pm using mist nets. When first captured, birds were ringed with a metal ring with a unique code, weighed, sexed and aged as juveniles (already moulted) or adults according to plumage patterns (Svensson 1992). Sex in robins, which are sexually monomorphic, was determined using genetic markers.

Birds were submitted to the experiments on the same day that they were captured. We used an enclosed artificial environment (a field tent), as testing arena, to measure the interactions between the pair of opponents. The tent size (base: 1.40 x 1.40 m, height 2.70 m; Appendix II) can be considered suitable for our experiments taking into account similar studies which used much-smaller arenas relatively to the body-size of birds (Pryke & Griffith 2006; Hasegawa et al. 2014). The behaviour of individuals was recorded using a video camera (HDR-CX220, Sony) fixed to the tent's top in a central position. We used three artificial wood trees with 1.70 m height (Fig. 3.1; Appendix II), like most plants with fleshy fruits available in the study area (1.60 ± 0.93 m, n = 35), and placed equidistantly at 0.60 m from each other. We tried to deal with potential individual variation in the height of foraging and type of food item. Thus, two feeders of

a randomly selected tree were filled with food (Fig. 3.1; Appendix II). Feeders were provided with 15 mealworms (total ca. 2 g) and 3 dark-red grapes (ca. 1.5 cm diameter) allowing individuals to choose between items suitable for both species (Cramp & Perrins 1994; Tsurim et al. 2008; Campos et al. 2011a). We conducted preliminary experiments with individual birds to verify if 10 minutes was a suitable period to explore the available space and find the food. Since only one individual (among 15 preliminary experiments) did not feed neither perched near the food, we considered that 10 minutes was a suitable period to motivate food defence. Therefore, to promote the occurrence of aggressive interactions, the two birds were introduced in the testing arena in two steps differing by 10 minutes (henceforth the first-introduced and the second-introduced individuals, respectively). Each bird was released by hand inside the tent through the door at a 0.50 m height. The experiments started 5 seconds after the release of the second individual and lasted for 10 minutes. No individual was used in more than one experiment. We minimized the effect of body size in the aggressiveness of birds by using pairs of opponents with similar weight (a paired T-test showed no significant differences in the body-weight of opponents: $t = -0.214$, $df = 62$, $p = 0.83$). No birds were injured during handling and experiments. During experiments, no fights were recorded and attack behaviour always led to opponent displacement without injuries. All individuals were released immediately after the experiments.

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Top: 1.70 m

1st branch: 1.55 m

2nd branch: 1.35 m

wood stick support
tree feeder

3rd branch: 1.10 m

4th branch: 0.65 m

5th branch: 0.20 m

wood stick support
ground feeder

Ground: 0 m
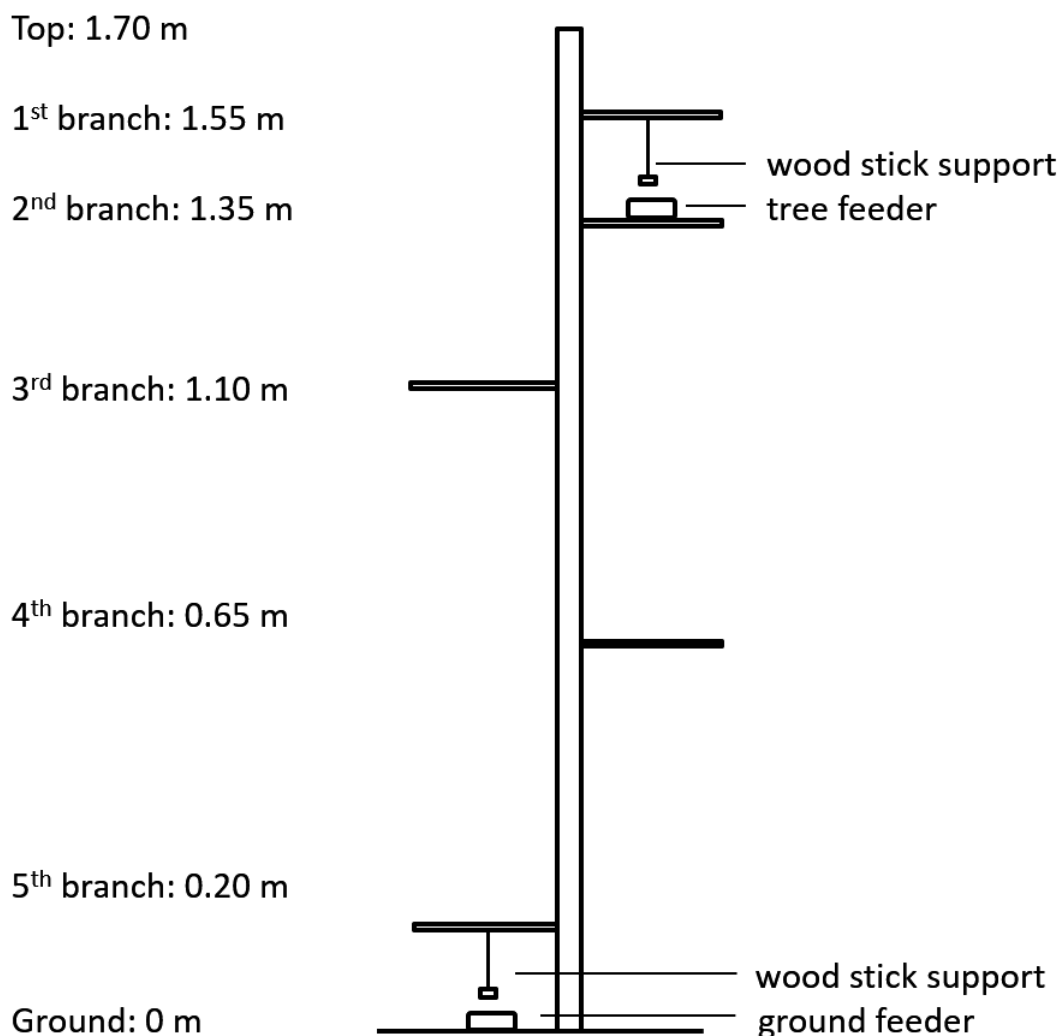
Fig. 3.1: Scheme of an artificial tree used in the experiments inside the tent showing five parallel branches (0.30 m long), wood stick supports with three wood pegs where grapes were suspended (stick plus pegs had 14 cm total length) and feeders where mealworms were placed (plastic light-brown coloured, height: 2.5 cm; basal section: 6 x 6 cm). For details see Appendix II.

Blood collection and sexing of robins

A drop of blood was collected (by PFP) from each robin by puncturing the brachial vein. Sexing was done by amplification of a fragment of the CHD gene by polymerase chain reaction (PCR), using the primers P2 and P8 (Griffiths et al. 1998). The primer P2

was labelled with the fluorescent dyes FAM. PCR amplification with Mytaq (Bioline) was performed in a 10 µl reaction mixture following the manufacturer's protocol. Amplification was conducted in a T100 Biorad thermal cycler under the following conditions: 95 ºC for 5 min, 28 cycles at 94 ºC for 30 sec, 48 ºC for 45 sec and 72 ºC for 45 sec, and further extension at 72 ºC for 5 min. The PCR products were separated by capillary electrophoresis on an automatic sequencer ABI 3130xl Genetic Analyzer. Fragments were scored against Genescan-500LIZ size Standard, using GeneMapper version 4.1 (Applied Biosystems). The sex determination process was done at the CTM – Centro de Testagem Molecular of the Research Centre in Biodiversity and Genetic Resources (CIBIO).

Behavioural observations

We performed a total of 63 experiments divided into treatment conditions based on species, sex and age of both opponents. Using the first-introduced individual as reference, we obtained conspecific experiments (blackcap-blackcap: n = 16; robin-robin: n = 13), heterospecific experiments (blackcap-robin: n = 17; robin-blackcap: n = 17), same sex experiments (female-female: n = 5; male-male = 24), different sex experiments (female-male: n = 21; male-female: n = 13), same age experiments (juvenile-juvenile: n = 21; adult-adult: n = 13), and different age experiments (juvenile-adult: n = 16; adult-juvenile: n = 13). The videos were analysed using the software OBSERVER 9 (Noldus Information Technology, Wageningen, The Netherlands).

We recorded the frequency and latency of social interactions between the opponents (both non-aggressive and aggressive behaviour), to analyse the behavioural relevance of heterospecific aggressiveness compared with conspecific aggressiveness (Appendix III). The identity of each opponent in every interaction was accurately determined as we

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managed to follow the movements of the first-introduced individual all the time. Social behaviour was recorded in reference to the first-introduced individual. We distinguished three types of behaviours with different degrees of aggressiveness (adapted from Thompson 1960; Senar 1990): i) lower-intensity head forward display (D1): when a bird remains still with the neck partially extended while pointing the bill towards the other; ii) higher-intensity head forward display (D2): when a bird opens or snaps the bill near the opponent with the neck fully extended, wings and tail slightly open; iii) attack: when a bird makes a fast movement towards the opponent using the legs or the bill as weapon, which results in physical contact between both individuals unless the victim escapes before the contact. In each experiment, we measured the frequency of each type of aggressive behaviour. We measured the latency to approach the opponent (in seconds), consider the time since the start of the experiment until the first aggressive behaviour of the first-introduced individual towards the opponent using a head forward display or an attack. Preliminary observations allowed us to make some decisions about the analyses of behaviours: (1) we excluded the non-aggressive behaviours from the analysis (clumping of individuals in four blackcap conspecific experiments), which constituted only 5.1% of all social interactions of blackcaps (n = 272) and were not recorded in robins (n = 323); (2) we focused only on the actions of the first-introduced individual as aggressor because there were no differences between the frequencies of aggressive behaviours when it was the aggressor or the target of aggression (generalized linear mixed model using the number of aggressive behaviours as response variable, the individual as explanatory variable and the experiment as random factor: t = -0.219, standard error = 0.218, df = 62, p = 0.828).

Data analysis

We computed four generalized linear models (GLM) using the treatments combining the "species", "sex" and "age" of opponents (three categorical variables with four levels) as explanatory variables in all models. Sex and age were selected to evaluate social dominance. We used as response variables: (1) the latency (in seconds) for the first aggressive behaviour when approaching the opponent (*latency to approach analysis*); (2) the number of D1 displays (*D1 displays analysis*); (3) the number of D2 displays (*D2 displays analysis*); and (4) the number of attacks (*attacks analysis*). The sample size differed slightly between analyses, since we excluded the experiments without approaches. The latency to approach was normally distributed. However, the frequencies of D1 displays, D2 displays and attacks contained more zeros than expected based on Poisson or negative binomial distributions. Considering that zero-inflated distributions affect the relationship between behavioural frequencies and "species", "sex" and "age" treatments, we analysed the data using zero-inflated Poisson and zero-inflated negative binomial models (Zuur et al. 2009; Bolker et al. 2012). For each dependent variable (D1 displays, D2 displays and attacks), we computed three zero-inflation GLM (models with a constant zero-inflation value only) differing in its family (Poisson, negative binomial - NB1 and NB2 parameterizations; Bolker et al. 2012). We selected the family considering the model with the lowest AIC, which for all three response variables was the zero-inflation negative binomial GLM (NB1 parameterization). Model results were validated using diagnostic plots. Pairwise comparisons of behavioural frequencies within treatments were computed with the non-parametric post-hoc Dunn's test after a Kruskal-Wallis test. All analyses were performed using the software R 3.3.0 (R-Core-Team 2016) with the packages

glmmADMB (Bolker et al. 2012), dunn.test (Dinno & Dinno 2015) and ggplots2 (Wickham 2016).

Results

Robins and blackcaps showed aggressiveness towards opponents in 80% and 73% of the experiments, respectively. Both species showed heterospecific aggressiveness. The frequency of D1 displays and attacks were different according to the "species" (Table 3.1; Fig. 3.2.b and Fig. 3.2.d). While, the latency to approach and the frequency of D2 displays were not affected by the "species" (Table 3.1; Fig. 3.2.a and Fig. 3.2.c).

In conspecific experiments, blackcaps used D1 displays less than robins (Dunn's test: $z = -1.999$, $p = 0.023$). Also, blackcaps attacked the opponents less frequently than robins (Dunn's test: $z = -1.875$, $p = 0.030$). However, robins seemed to use D1 displays more against conspecifics than against heterospecifics (Dunn's test: $z = -1.644$, $p = 0.050$). Robins attacked more their conspecifics than heterospecifics (Dunn's test: $z = -2.034$, $p = 0.021$). While blackcaps used D1 displays with similar frequency regardless the opponent species (Dunn's test: $z = 0.457$, $p = 0.324$). Also, in blackcaps, there were no differences in the attack frequencies between conspecifics and heterospecifics (Dunn's test: $z = -0.532$, $p = 0.297$).

Table 3.1: Results of the GLM for the *latency to approach analysis* and the zero-inflation GLMs (negative binomial) for *D1 displays*, *D2 displays* and *attacks* analyses, testing the effect of "species", "sex" and "age" treatments on the aggressive behaviour of the first-introduced individual towards the opponent ("species" treatments include European robins (R) and/or blackcaps (B); "sex" treatments include males (M) and/or females (F); "age" treatments include adults (A) and/or juveniles (J); SE - standard error; NB - negative binomial dispersion parameter; ZIP - zero-inflation parameter).

variable	Latency to approach				D1 Displays			
	Estimate	SE	test value	P	Estimate	SE	test value	P
Intercept	282.28	139.08	2.030	0.052	0.523	0.586	0.89	0.372
BxR	-56.79	83.46	-0.681	0.502	-0.199	0.396	-0.50	0.615
RxB	82.12	76.09	1.079	0.289	0.101	0.372	0.27	0.786
RxR	-13.51	77.97	-0.173	0.864	0.786	0.344	2.29	0.022
FxM	-12.73	136.37	-0.093	0.926	-0.036	0.635	-0.06	0.955
MxF	-22.73	137.69	-0.165	0.870	0.817	0.587	1.39	0.164
MxM	37.94	126.56	0.300	0.767	0.775	0.573	1.35	0.176
AxJ	-11.07	90.28	-0.123	0.903	-0.406	0.399	-1.02	0.310
JxA	-117.55	77.72	-1.512	0.141	-0.197	0.381	-0.52	0.606
JxJ	-172.99	71.63	-2.415	0.022	0.099	0.336	0.30	0.767
Model parameter	AIC				NB (SE)	ZIP (SE)	Log-likelihood	AIC
	517.57				3.795 (0.882)	1.102e-06 (<0.001)	-136.794	297.59

variable	D2 Displays				Attacks			
	Estimate	SE	test value	P	Estimate	SE	test value	P
Intercept	-17.733	4553.6	0.00	0.997	0.172	1.029	0.17	0.867
BxR	0.749	0.562	1.33	0.182	-0.016	0.768	-0.02	0.983
RxB	-0.628	0.675	-0.93	0.352	-0.221	0.880	-0.25	0.801
RxR	-1.906	1.154	-1.65	0.099	1.888	0.731	2.58	0.010
FxM	17.586	4553.6	0.00	0.997	-0.685	1.223	-0.56	0.575
MxF	18.754	4553.6	0.00	0.997	-1.593	1.426	-1.12	0.264
MxM	17.627	4553.6	0.00	0.997	0.102	1.083	0.09	0.925
AxJ	-0.334	0.816	-0.41	0.682	0.323	0.730	0.44	0.658
JxA	-0.684	0.772	-0.89	0.376	-0.894	1.183	-0.76	0.420
JxJ	-0.124	0.649	-0.19	0.848	-0.735	0.516	-1.43	0.154
Model parameter	NB (SE)	ZIP (SE)	Log-likelihood	AIC	NB (SE)	ZIP (SE)	Log-likelihood	AIC
	2.655 (0.984)	0.113 (0.175)	-61.111	146.22	1.634 (0.497)	0.400 (0.138)	-57.706	139.41
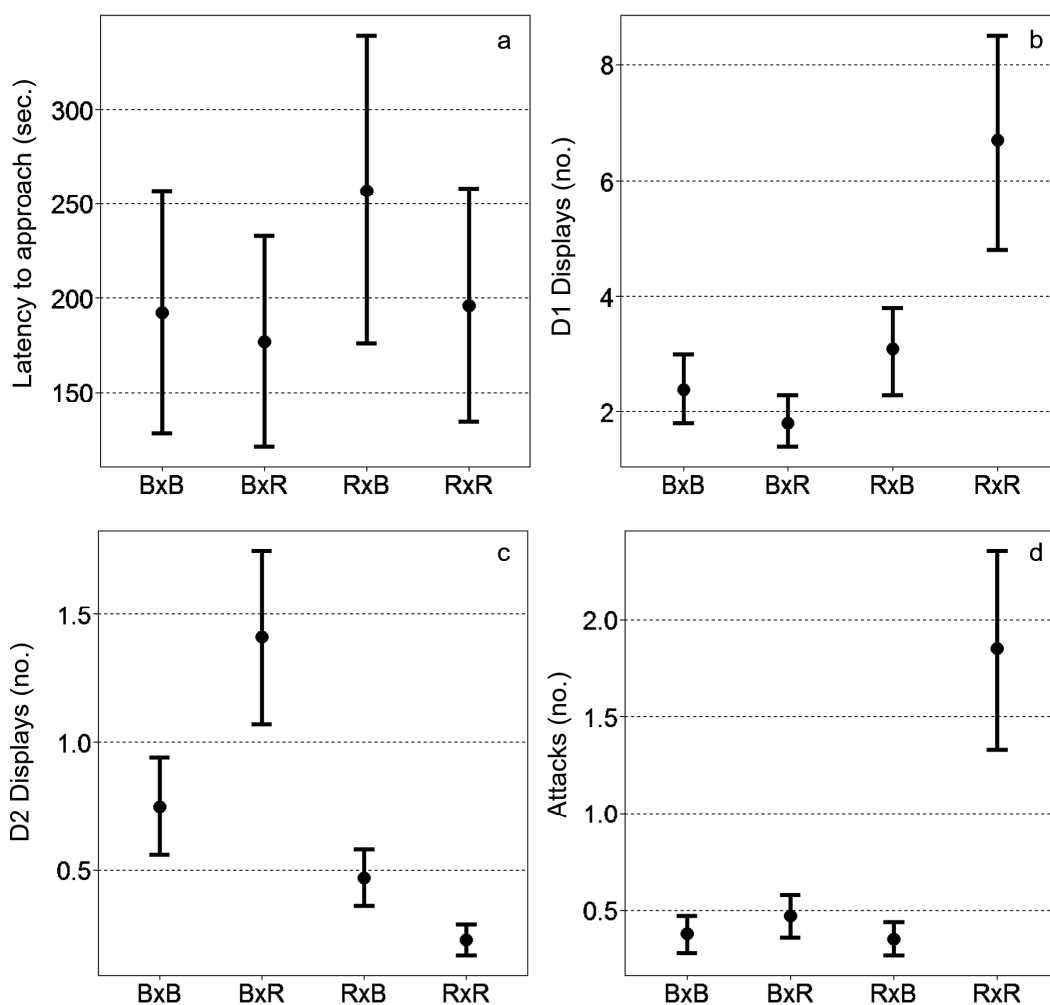
Fig. 3.2: Mean latency to approach the opponent (a) and mean frequency of aggressive behaviours (b-d) of the first-introduced individual towards the opponent. Bars denote the standard error. Data are shown according to the "species" treatments using a scheme of first-introduced individual vs second-introduced individual (B - blackcap; R - European robin).

Discussion

Our experiments simulating a competitive feeding context between robins and blackcaps outside the breeding season showed that they frequently engage in aggressive interactions (including both displays and attacks). Next, we present the arguments that

suggest that these interspecific aggressive interactions, besides being frequent in experimental controlled conditions in our study, may also be common in nature. The frequency of aggressive behaviour observed in our study can be particularly relevant considering that the individuals were in a neutral arena which, albeit maximizing the proximity between them, places them outside their territories (and the levels of aggressiveness should be higher inside than outside the territories; Dingemanse & de Goede 2004). We observed several body postures of typical aggressive displaying and attacking behaviour of these species such as described in literature (Cramp & Perrins 1994), which indicate that their behaviour was not affected by the artificial setup.

The latency of aggressive approach appears to indicate that robins and blackcaps did not ignore each other and often behaved agonistically. First, the large differences in plumage between our species excludes the possibility of rival misidentification (Martin et al. 1996; Toms 2013). Second, the absence of differences in latency to agonistic behaviours between species can be an indication of heterospecific rival recognition (Matyjasiak 2005; Grether et al. 2009; Peiman & Robinson 2010). The two species co-occur widely and they have similar use of resources, so that rival recognition between them is highly likely such as occur with other species with similar ecologies (Hansen & Slagsvold 2003; Matyjasiak 2005; Jaška et al. 2015).

Conspecific aggressiveness was more intense than heterospecific aggressiveness in robins but not in blackcaps when considering the frequency of D1 displays and attacks. The observed differences in agonistic behaviour between the two species may be due to the fact that robins are territorial in winter grounds, whereas blackcaps forage opportunistically and do not incur costs of territorial defence. The defence of winter territories is an adaptive strategy of robins, which may contribute to provide suitable

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sheltering and more food resources, and therefore improving the mating chances in the case of year-round territories (Adriaensen & Dhondt 1990; Cuadrado 1997). By defending non-breeding territories, robins maintain a high singing effort and frequent chases of conspecific neighbours, with a consequent reduction of the time available for foraging activities (Tobias 1997). With these added costs, it is expected that robins should direct their aggression to conspecifics which pose a greater threat. Similar results were obtained in other studies with sexually monomorphic passerines with year-round territories (Tobias et al. 2011; Toms 2013). According to Tobias et al. (2011) the defence of year-round territories mostly towards conspecifics can be indicative of a great intrasexual competition, which appears to occur also in robins, since they are also sexual monomorphic and both sexes sing and defend territories outside the breeding season (Schwabl 1992; Tobias 1997).

Contrary to our expectations (Pryke & Andersson 2003; Gauvin & Giraldeau 2004), blackcaps were aggressive during interactions. Conspecific aggression in non-territorial species during the non-breeding period is generally a result of differential dominance status of individuals within their social hierarchies (Senar 1990; Funghi et al. 2015b; Leitão et al. 2015). In feeding contexts, these interactions are most likely among gregarious species, which is not the case of the blackcap (Leach 1981; Senar 1990; Taillon & Côté 2007). Recently, Morganti et al. (2017) found that in some blackcaps the space which is defended for breeding is often included within their winter home-ranges (on average six times larger in winter than during breeding). These authors suggested that such spatial arrangement may motivate some aggressiveness towards conspecifics. However, social dominance among blackcaps is unclear (inferred from the habitat-type used) or absent and thus its putative effect on conspecific aggressiveness should be low (Leach 1981; Pérez-Tris & Tellería 2002b; Morganti et al. 2017). Dominance in

blackcaps may also result from other traits which were not analysed here, such as badges of status, e.g. cap characteristics (Pryke & Griffith 2006). Our results indicated that blackcaps were equally aggressive towards conspecifics and heterospecifics. Since higher resource overlap (e.g. feeding resources) may evoke higher aggressiveness between opponents, such similarity suggests that competition should be equivalent at both conspecific and heterospecific levels (Muñoz & Motta 2000; Peiman & Robinson 2010; Ferretti et al. 2011). In our case, the heterospecific aggressiveness of blackcaps towards robins can be related with resource limiting. Winter conditions in deciduous forests should concentrate the food resources in scrubs (fruit and invertebrate resources) and on the ground (invertebrates; Carrascal et al. 1987; Hernández 2008). However, blackcaps forage only on scrubs, while robins also on the ground (Jordano 1982; Carrascal et al. 1987; Cuadrado 1997). Therefore, winter resources appear to be more limiting for blackcaps, which can motivate their aggressiveness towards robins (Pérez-Tris & Tellería 2002a; Hernández 2008). Considering that the mechanisms to achieve dominance can differ between conspecifics and heterospecifics (Brazill-Boast 2013), additional experiments testing the heterospecific dominance between robins and blackcaps are needed.

Our study provides evidence supporting the existence of interspecific aggressive behaviour among two species belonging to different families of birds. Similarities in latency to approach the opponent, either a conspecific or heterospecific, suggest heterospecific-rival recognition. In fact, although differing in territorial behaviour, robins and blackcaps, when competing for food, deal with conspecifics and heterospecifics with aggressiveness rather than with non-aggressive social behaviour. We also show that robins discriminate between conspecifics and heterospecifics, being

more aggressive towards the first. Conversely, blackcaps are equally aggressive regardless the opponent species. Therefore, it is worthwhile investigating heterospecific aggressiveness even in phylogenetically-distant species, because it may be an important mechanism acting on its natural populations. In future studies, it would be interesting to use an optimality approach to obtain a more quantitative measure of the degree of interspecific competition, by manipulating resource defensibility and abundance.

Acknowledgements

Chapter 4 – Competitive advantages of the red-billed leiothrix (*Leiothrix lutea*) invading a passerine community in Europe

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Abstract

The establishment of an introduced species is an important step of the invasion pathway. Often species become established through their superior competitiveness over the native community or by occupying empty niches. Recently, the red-billed leiothrix *Leiothrix lutea* has become established in some European natural-woods, which can be quite relevant for nature conservation considering its position among the seven exotic bird species with highest negative impact in bird communities. We assessed which European-native species are more likely to compete (i.e. potential competitors) with leiothrix based on their structural size and diet composition. Also, we evaluated the competitive advantages of leiothrix relatively to its potential competitors that may allow its successful establishment considering two approaches: exploratory behaviour and foraging morphology. Four species showed great similarity in structural size with the leiothrix, and two of them also presented great similarity in diet composition, which makes them potential competitors: the robin *Erithacus rubecula* and the blackcap *Sylvia atricapilla*. The exploratory behaviour of the leiothrix did not differ from those of its potential competitors. However, the leiothrix presented more efficient foraging morphology than their potential competitors. Our results support the hypothesis of an establishment process by competition over native species rather than an opportunistic occupation of an empty ecological niche. The establishment of the leiothrix in European natural-habitats, and not in highly disturbed habitats as other invasive species, may constitute a new challenge for the native species conservation.

Keywords: Competition Hypothesis, *Erithacus rubecula*, establishment, Opportunistic Hypothesis, *Sylvia atricapilla*

Introduction

Will the newcomer become a pest? This is probably the most important and more frequently asked question in invasion ecology (Nentwig 2007; Lockwood et al. 2013). Invasion ecology research has given evidence that the interactions between the introduced species and the receiving native community may play a fundamental role in determining the outcome of the invasion (Shea & Chesson 2002; Duncan et al. 2003). The introduction of a species in a non-native environment allows studying the interaction with the native community and the role played by long coevolved processes within the native community, such as interspecific competition (Petren & Case 1996; Duncan 1997; Holway 1999).

The establishment of an introduced animal species in the new environment is an important step of the invasion pathway which determines its spreading potential (Duncan et al. 2003). Some characteristics of successful colonizers can be regarded as pre-adaptations for their establishment (Sol 2008; Chapple et al. 2012). Therefore, a relevant question becomes central when facing the presence of a newcomer: does this species have characteristics that confer it an advantage for establishing in the new environment? Distinct ecological niches and greater exploratory abilities of an introduced species when compared with native species have been suggested as mechanisms for the establishment success (Sol et al. 2002; Chapple et al. 2012; Batalha et al. 2013; Azzurro et al. 2014; Sanz-Aguilar et al. 2015). The occupation of an empty ecological niche by an introduced species can allow its establishment without competition with native species, a process designated as "opportunistic hypothesis" (Shea & Chesson 2002; Sol et al. 2012; Batalha et al. 2013; Azzurro et al. 2014). Alternatively, when ecological niches of introduced species and native species overlap,

they will often compete for the same resources and thus the establishment of the introduced species is dependent on its superior competitiveness, a process designated as "competition hypothesis" (Petren & Case 1996; Holway 1999; Foster & Robinson 2007; Sol et al. 2012; Pejchar 2015). Such superior competitiveness can result from a greater foraging efficiency and exploratory ability of the introduced species when compared with native species (Petren & Case 1996; Piet 1998; Rehage & Sih 2004).

Birds are suitable models to study animal invasions as (1) they are a well-studied group, particularly in terms of ecology and behaviour; (2) have great mobility which can improve their spreading rate; and (3) are responsible for several kinds of negative impacts in introduced communities, including competition with native species (Duncan et al. 2003; Martin-Albarracin et al. 2015). However, the current knowledge on the effects of introduced species in natural habitats (where the impact in native communities should be greater than in highly disturbed habitats) is still limited (Sol et al. 2012; Martin-Albarracin et al. 2015). The red-billed leiothrix (*Leiothrix lutea*, F. Timaliidae; hereafter leiothrix) is a small passerine native to south-eastern Asia, and without closely related species in Europe. In the last decades, their popularity as a cage-bird has resulted in several successful introductions in natural woodlands in different regions across the world, such as Hawaii, Japan, and more recently in Europe (Fisher & Baldwin 1947; Amano & Eguchi 2002; Puglisi et al. 2009; Catry et al. 2010; Herrando et al. 2010; Herrando et al. 2011; Dubois & Cugnasse 2015). The species was first recorded in the wild in central Portugal during 2007 in a remote region rarely visited by birdwatchers (Matias 2010). A population has become established in this region, increasing in area and density, and has been estimated to reach 500-700 pairs in 2016 (PFP, unpublished data). Recently, a worldwide-scale meta-analysis with avian introductions considered

the leiothrix among the seven bird species with highest negative impact in bird communities (including competition with native species and disease introduction; Martin-Albarracin et al. 2015). The leiothrix is a suitable model to study the mechanisms of invasion success in native communities due to: (1) its preference for natural habitats, such as rich and dense woodlands, that are refuges for native woodland bird species in regions highly fragmented by human alteration of the natural landscape (Herrando et al. 2010; Farina et al. 2013); (2) strong evidence of niche conservatism of the leiothrix both in its native and European populations, suggesting high environmental matching between its native and non-native distribution (Strubbe et al. 2013); (3) its generalist diet, which includes fruits and invertebrates (Foster & Robinson 2007; Pejchar 2015); (4) its relatively extended breeding season, which extends from April to August in central Portugal (PFP, unpublished data) ; (5) its gregarious habits outside the breeding season (PFP, unpublished data; Vall-llosera et al. 2016) which can improve the exploratory capability and competitive dominance when compared to solitary species with similar feeding habits; and (6) its great success as invasive species attested by the high relative abundances among invaded communities in several continents (Amano & Eguchi 2002; Foster & Robinson 2007; Farina et al. 2013; Pejchar 2015; Vall-llosera et al. 2016).

Despite the suggestion that competition exists between the leiothrix and European-native passerines, its competitiveness potential remains unknown (Herrando et al. 2010; Farina et al. 2013; Martin-Albarracin et al. 2015). The aims of our study are (1) to identify which European-native species are more likely to compete with the leiothrix (defined here as potential competitors), and (2) to evaluate possible competitive advantages of the leiothrix relatively to its potential competitors, which may contribute

to the invader establishment success. First, we assessed the potential competitors based on structural size differences and diet composition, which are suitable indicators of potential competition (Palmeirim et al. 1989; Azzurro et al. 2014). Second, we evaluated the exploratory behaviour and the foraging morphology of the leiothrix and their potential competitors, which are two useful approaches to measure competitive advantage (Rey & Gutiérrez 1996; Rehage & Sih 2004). The leiothrix can be considered a better competitor than its potential competitors if it has higher exploratory behaviour or more efficient foraging morphology.

Methods

Study area

The study was performed in two areas in central Portugal. Data for the leiothrix and other small woodland insectivorous passerines (hereafter native passerine community) were obtained near Miranda do Corvo (area 1: 10 ha; ca. 175 m a.s.l.; 40° 03'N, 8° 20'W) in a small stream valley bordered by a dense wood with diverse deciduous trees (oak *Quercus robur*, willows *Salix* spp., chestnut trees *Castanea sativa* and poplar *Populus nigra*) and a dense scrub cover, mostly composed by brambles (*Rubus* spp.). We also collected data from passerines captured in the constant-effort ringing site of Évora University located in Herdade da Mitra (area 2: 6 ha; ca. 220 m a.s.l.; 38° 31'N, 8° 01'W; 170 km from area 1) where the landscape is composed by several woodland habitats.

Bird Captures

Field work was performed between May 2014 and April 2015 in area 1 and between February 2010 and December 2015 in area 2. Birds were captured using mist nets and when first-captured were ringed using a metal-ring with a unique alpha-numerical code.

We focused our study to sympatric native species with a similar diet (insectivorous-frugivorous diet) and similar weight (10-30 g), as these are the species more likely to compete with the leiothrix. To avoid putative bias caused by incomplete size development, we excluded from analysis all first-year individuals until October. Data from area 2 was used to increase sample size (i.e. number of individuals) of the structural size analysis. The exploratory behaviour tests and foraging morphology analyses were performed just for the leiothrix and their potential competitors using data from area 1.

Identification of potential competitor species

The determination of potential competitors of the leiothrix resulted from two crossed criteria: (1) we found which species had greater similarity in structural size with the exotic species; (2) we found which species have a similar diet composition to the leiothrix from specialized literature (Herrera 1984; Cramp & Perrins 1994; Fuentes 1994). For calculating the structural size index of species from the native passerine community, we collected three biometrical data for first-captured individuals from both areas following Svensson (1992): maximum length of wing (±1 mm) from the carpal joint to the longest primary with wing closed using a ruler with a zero-stop; length of bill to skull (±0.1 mm); and tarsus length from the notch of the intertarsal joint to the

lower edge of the last complete scale before the toes diverge (±0.1 mm) using a calliper. We considered that native species which have a mixed insectivorous-frugivorous diet (> 50 % faecal samples with seeds) are more likely to compete with the leiothrix, which can have at least 84 % of faecal samples with seeds (Corlett 1998; Foster & Robinson 2007; Pejchar 2015).

Exploratory behaviour

We conducted an enclosure experiment between November 2014 and February 2015 in order to detect differences in exploratory behaviour between the leiothrix and its potential competitors. We randomly selected 15 different leiothrix individuals and the same number of those potential competitors to perform the experiment. Individuals were evenly distributed according to age and sex in order to control for their possible effects. Tests were adapted from Dingemanse et al. (2002) and were conducted using one individual inside a field tent (height 2.70, basal section: 1.40 x 1.40 m; Appendix II) which was opaque but not sound isolated from the exterior. Each bird was released by hand inside the tent through the door at a 0.50 m height and the test started after 5 seconds. Behavioural observations were conducted for 10 minutes and recorded using a small video camera (HDR-CX220, Sony) fixed to the tent roof in a central position. To replicate a naturalized environment, we used three artificial wood trees with 1.70 m height with five parallel branches of 0.30 m long, located at different heights. Video recordings were analysed using the software OBSERVER 9 (Noldus Information Technology, Wageningen, The Netherlands). We recorded the number of occurrences of three exploratory behaviour types: ground jumps (movements on the ground), branch jumps (movements within the same branch) and flights between branches. All

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experiments were performed under permits from national authorities which allow bird trapping, ringing, manipulation and experiment (Portuguese National Authority for Animal Health (DGAV) and Portuguese National Institute for Nature Conservation and Forests (ICNF)). Ringing licences were attributed to P.F.P (185/2014) and to C.G. (36/2015) by ICNF. The birds were released immediately after the tests. No birds were injured during handling or experimentation.

Foraging morphology

For the first-captured individuals of the leiothrix and its potential competitors, we collected morphological data related with foraging ecology: gape width (±0.1 mm) of mouth commissures with the bill closed measured using a calliper; and length of each primary (±1 mm) of the right wing measured using a pin-ruler. At the time of captures, none of the measured birds was in active moult of flight feathers. The gape width was used as an indicator of specialization in frugivory because larger gapes improves fruit handling and swallowing (Rey et al. 1997; Carnicer et al. 2009). In southern Europe, frugivory is a relevant trophic strategy for survival during the seasonal scarcity of insects, which occurs mainly in winter (Carnicer et al. 2009). The length of primaries was used to evaluate the wing roundness as an indicator of specialization for woodland dwelling (i.e. rounded wings improve dwelling in dense habitats; Morrison 1982; Keast 1996; Dawson 2005). We tried to exclude the putative effect of migratory behaviour of birds in its wing shape (Gaston 1974; Yong & Moore 1994; Keast 1996; Kaboli et al. 2007) by classifying the individuals as migrants/vagrants (if captured only in autumn/winter) or residents (if captured also in spring).

Data analysis

All variables were normally distributed, except for exploratory behavioural variables (number of ground jumps, branch jumps and flights between branches) which were square-root transformed. First, we analysed bird structural sizes in order to find which native-species are more likely to compete with the leiothrix. The length of wing, bill and tarsus were collected for 12 species of passerines in both study areas (Table 4.1). We performed a principal component analysis (PCA) with the three variables in order to reduce them to a structural size index (first PCA component) which can be regarded as an indicator of general morphology and size (Fernández-González et al. 2013).

Table 4.1: Acronyms (Acro.), taxonomic family, sample size (n) and biometric data of small insectivorous woodland passerines and the results of linear regression comparing the structural size between the leiothrix and other species using the first component of a PCA with wing, bill and tarsus length. Measurements given in mm (mean ± standard deviation).

Species	Acro.	Family	n	Wing length	Bill length	Tarsus length	t-value	P
Leiothrix (*Leiothrix lutea*)	Llut	Timaliidae	75	68.4 ± 2.0	15.7 ± 0.6	24.9 ± 0.9	-	-
Robin (*Erithacus rubecula*)	Erub	Muscicapidae	164	70.7 ± 2.2	14.6 ± 0.6	25.2 ± 0.8	-1.666	0.096
Blackcap (*Sylvia atricapilla*)	Satr	Sylviidae	386	71.3 ± 2.1	14.7 ± 0.7	20.6 ± 0.7	-19.009	<0.001
Dunnock (*Prunella modularis*)	Pmod	Prunellidae	11	66.3 ± 3.3	14.1 ± 0.8	19.9 ± 0.7	-13.681	<0.001
Nightingale (*Luscinia megarhynchos*)	Lmeg	Muscicapidae	51	80.6 ± 2.5	17.0 ± 0.7	27.1 ± 0.8	28.251	<0.001
Cetti's warbler (*Cettia cetti*)	Ccet	Sylviidae	12	57.7 ± 3.3	14.3 ± 0.7	20.6 ± 0.9	-18.982	<0.001
Great tit (*Parus major*)	Pmaj	Paridae	34	71.0 ± 2.2	12.9 ± 0.6	19.5 ± 0.7	-22.274	<0.001
Sardinian warbler (*Sylvia melanocephala*)	Smel	Sylviidae	73	58.4 ± 1.5	13.9 ± 0.6	19.9 ± 0.8	-38.878	<0.001
Chaffinch (*Fringilla coelebs*)	Fcoe	Fringillidae	67	82.8 ± 3.6	14.3 ± 0.8	18.0 ± 0.7	-7.975	<0.001
Nuthatch (*Sitta europaea*)	Seur	Sittidae	22	80.7 ± 2.0	19.4 ± 0.8	19.0 ± 0.5	12.511	<0.001
Crested tit (*Lophophanes cristatus*)	Lcri	Paridae	11	59.5 ± 2.2	11.4 ± 0.6	17.8 ± 0.7	-29.053	<0.001
Blue tit (*Cyanistes caeruleus*)	Ccae	Paridae	144	59.0 ± 2.2	9.2 ± 0.6	15.9 ± 0.8	-86.704	<0.001

We used the first PCA component in a linear regression (LR) to measure the differences between the structural size of the leiothrix and the native passerine community. We used the centroids of each species in the PCA to measure the morphological distance (Euclidean distance) between native species and the leiothrix. The potential of competitiveness between species increases inversely to their respective morphological distance (Batalha et al. 2013; Azzurro et al. 2014). In this study, we considered as direct competitors of the leiothrix the species which showed less than 50 % of the maximum observed of Euclidean distance to it. We used the results of the structural size index analysis to select the number of species to be used in the following analyses (exploratory behaviour and foraging morphology).

We reduced the three behavioral variables (ground jumps, branch jumps and flights between branches) with a PCA and used its components as response variables in a LR, having the species as explanatory variable. With this procedure, we measured the variation in exploratory behaviour between species. The leiothrix can be considered a better competitor than its potential competitors if it has better exploratory skills (by having a higher total of occurrences).

To analyse differences in foraging morphology between species, we performed two LR using gape width and wing shape as response variables. The species was used as explanatory variable in both LR, and in the second LR we also included the migratory behaviour of individuals in order to control its effect on wing shape. We first reduced the variables describing primaries length with a PCA. The first component, which can be used as indicator of wing shape (Swaddle & Lockwood 2003; Peiró et al. 2006; Andrade et al. 2015), was used as response variable in the LR. The leiothrix can be considered a better competitor than its potential competitors if it has traits that suggest

higher efficiency in frugivory and forest-dwelling (by having a wider gape and more round-shaped wing). All analyses were performed using the software R 3.3.0 (R-Core-Team 2016) with the packages FactoMineR (Lê et al. 2008) and lsmeans (Lenth 2013).

Results

Identification of potential competitor species

The structural size index was different between the leiothrix and the species of the native passerine community except for the European robin (henceforth robin; t-value = -1.666, p = 0.096; Table 4.1). The first component of PCA describing a structural size index explained 71.42% of the overall variation and had a strong and positive correlation (p < 0.001) with the wing, bill and tarsus length (Appendix 4.1). When considering the Euclidean distances between the leiothrix and native species, the maximum was recorded for the blue tit (Fig. 4.1). Four native species showed greater similarity in structural size index with the leiothrix: robin, blackcap, dunnock and nightingale (Fig. 4.1). However, the dunnock and the nightingale have diets relying much less on fruits than the leiothrix, or the robin and the blackcap (faecal samples with seeds: 30 % for dunnock and nightingale and 84 % for leiothrix, robin and blackcap; Herrera 1984; Fuentes 1994). Therefore, we not included the dunnock and the nightingale in the following analyses and the species with a greater probability of competing with the leiothrix (here defined as its potential competitors) were the robin and the blackcap.
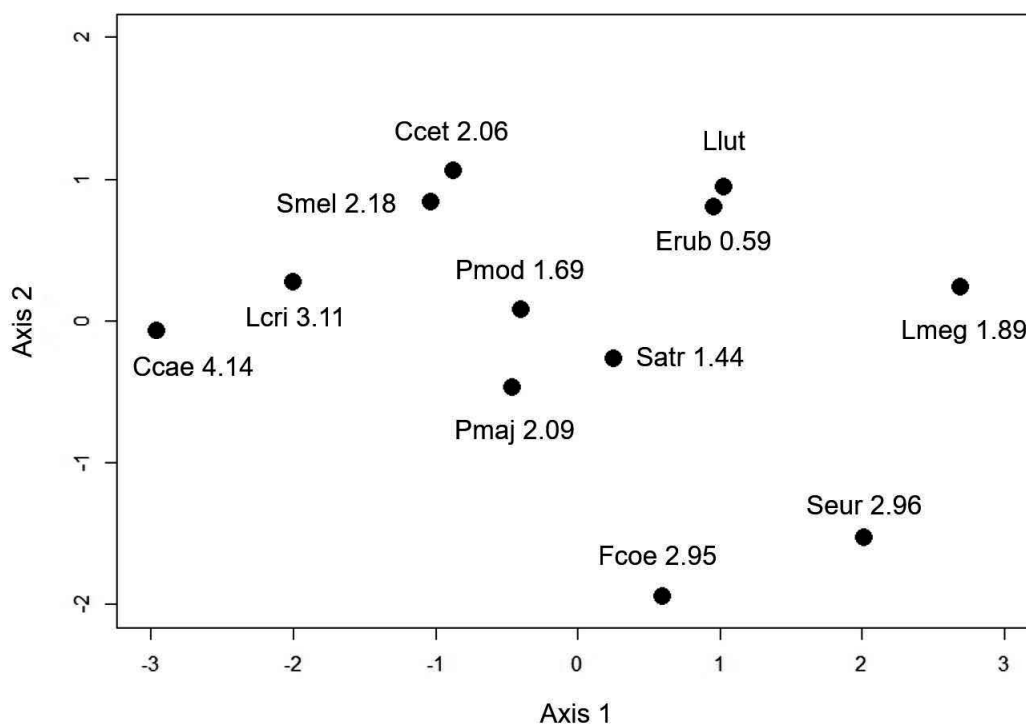
Fig. 4.1: Centroids of a Principal Component Analysis representing the structural size (with wing, bill and tarsus length) of small insectivorous woodland passerines and the morphological distance (Euclidean distance) between each species and the leiothrix. For species acronyms see Table 4.1.

Exploratory behaviour

The pattern of exploratory behaviour did not differ between the leiothrix and its potential competitors. The first component of PCA (exploratoryPCA1), which appears to be related with arboreal activity, explained 55.29% of the overall variation and presented a strong and positive correlation with branch jumps and flights (Appendix 4.2). The second component (exploratoryPCA2), which explained 33.28% of the overall variation, had a strong and positive correlation with ground jumps. Arboreal activity did not differ between the leiothrix and its potential competitors (robin: $t = -1.451$, $p = 0.154$; blackcap: $t = 1.828$, $p = 0.07$; Table 4.2). Similar results were obtained for ground activity (robin: $t = 1.692$, $p = 0.098$; blackcap: $t = -1.152$, $p = 0.256$; Table 4.2).

Table 4.2: Measurements of the exploratory behaviour, gape width and primaries length of the leiothrix, robin and blackcap (mean ± standard deviation). Here the first primary is the innermost.

Parameters		Leiothrix	Robin	Blackcap
Exploratory behaviour (counts during 10min.)	Sample size	15	15	15
	Ground jumps	19.1 ± 37.5	36.9 ± 46.2	3.1 ± 5.1
	Branch jumps	24.5 ± 36.2	8.1 ± 10.8	28.5 ± 23.3
	Flights	11.1 ± 11.6	14.7 ± 27.9	20.9 ± 16.5
Gape width (mm)	Sample size	52	82	118
		8.4 ± 0.3	7.6 ± 0.3	8.1 ± 0.4
Primaries length (mm)	Sample size	53	86	130
	1st P	53.5 ± 1.7	51.6 ± 1.7	48.7 ± 1.9
	2nd P	53.8 ± 1.8	52.3 ± 1.7	50.1 ± 1.8
	3rd P	56.1 ± 1.9	53.2 ± 1.8	51.1 ± 1.8
	4th P	57.9 ± 2.0	55.0 ± 2.0	52.1 ± 1.7
	5th P	57.8 ± 2.1	58.7 ± 2.2	53.8 ± 1.6
	6th P	56.7 ± 2.0	58.5 ± 2.2	56.6 ± 1.9
	7th P	54.9 ± 2.2	56.9 ± 2.0	57.3 ± 2.1
	8th P	50.2 ± 2.2	53.3 ± 2.0	55.7 ± 2.3
	9th P	39.2 ± 2.3	43.9 ± 1.8	49.1 ± 2.2
	10th P	22.1 ± 1.8	21.1 ± 1.7	16.0 ± 1.3

Foraging morphology

Foraging morphology differed between the leiothrix and its potential competitors. The mean gape width was significantly larger in the leiothrix than in the robin ($t = -14.280$, $p < 0.001$; Table 4.2), and also larger than in the blackcap ($t = -5.535$, $p < 0.001$; Table 4.2). The first component explained 58.45% of the overall variance and had a strong positive correlation ($p < 0.001$) with the length of the most proximal primaries (1st to 6th) and with the 10th primary, and a negative correlation with the length of the 8th and 9th primaries (Appendix 4.3), which can be described as an index of the wing pointedness (Peiró et al. 2006; Andrade et al. 2015). The leiothrix has less-pointed wings (i.e. more round-shaped) than the robin ($t = -4.24$, $p < 0.001$) and even less-pointed than the blackcap ($t = -12.55$, $p < 0.001$). The migratory behaviour of

individuals did not affect their wing shape (t = -0.172, p = 0.86). The higher values of gape width and greater wing roundness of the leiothrix may provide a competitive advantage over native species.

Discussion

The evidences gathered in our study suggest that the introduced leiothrix has a great potential for establishment within the community of small woodland insectivorous passerines in Portugal, and possibly in several other European countries as discussed by Martin-Albarracin et al. (2015). The leiothrix shows characteristics that make it a potentially successful invasive species, and in this particular case with a greater probability of competing with two native species: the robin and the blackcap. The establishment process of the leiothrix seems to be mostly facilitated by the fact that this species is a stronger competitor than their potential competitors, with evidences supporting the competition hypothesis in opposition to the opportunistic hypothesis. The leiothrix had a structural size index similar to the robin, which is not in agreement with the opportunistic hypothesis. Actually, the potentially more efficient foraging morphology of the leiothrix when compared with both potential competitors supports the competition hypothesis.

The opportunistic hypothesis predicts that an introduced species should occupy an "empty niche" within the receiving community (Shea & Chesson 2002; Sol et al. 2012; Batalha et al. 2013; Azzurro et al. 2014). Instead, the leiothrix position in the morphological space is similar to the robin and to a lesser extent also to the blackcap, dunnock and nightingale. This result suggests that the leiothrix may compete with these

native species rather than finding himself in an empty niche. Moreover, considering the frequency of exploratory behaviour, the leiothrix shows a similar and clearly overlapping pattern to the ones of the robin and the blackcap. This may suggest some degree of foraging competition among them (Schoener 1983; Petren & Case 1996). However, our results also suggested that, when exposed to the same novelty of an artificial environment, the leiothrix has no particular competitive advantage over the native species regarding their exploratory behaviour. Vall-llosera et al. (2016) using also a field experiment found that the leiothrix didn't shown more opportunistic behaviour than the natives too. This paper also suggests that the collective foraging could be a competitive advantage of the leiothrix over native species.

The foraging morphology analyses support the competition hypothesis for the establishment of the leiothrix. This species has a wider gape and more rounded wings than the robin and the blackcap. Observational field experiments as conducted by other authors (Amano & Eguchi 2002; Vall-llosera et al. 2016) could add more information about foraging niche overlap between these species rather than only studying their morphology. However, the morphological traits of the leiothrix, which were analysed in our study, are indicative of a more efficient foraging in woodlands when compared to the native species (Morrison 1982; Keast 1996; Rey et al. 1997; Dawson 2005; Carnicer et al. 2009). These characteristics may contribute to a dominant competiveness of the leiothrix over the native species, which can be quite relevant considering the ecological similarities between the three species. As an example, all three species shift their diet seasonally following the pattern of major fruit availability (Jordano 1982; Herrera 1998; Foster & Robinson 2007; Pejchar 2015). However, while robins and blackcaps developed several mechanisms, such as interspecific aggression and different fruit

capture techniques, to allow their coexistence while keeping similar fruit preferences (Jordano 1982; Rey et al. 1997; Herrera 1998; chapter 3), the leiothrix is a newcomer with unknown foraging abilities. Since gape width constrains the ability for fruit handling and thus foraging efficiency (Rey et al. 1997), the larger gape of the leiothrix possibly makes it a more efficient consumer than the robin and the blackcap, and thus a stronger competitor for food resources. Such prediction is particularly relevant since the leiothrix presents a diverse frugivorous diet, including novel fruit-species that are absent in its native-range and some fruit genera which are also selected by the robin and the blackcap (e.g. *Rubus* spp. and *Vaccinium* spp.; PFP, unpublished observation; Cramp & Perrins 1994; Corlett 1998; Foster & Robinson 2007; Pejchar 2015).

Considering the absence of a relation between wing-shape and migratory behaviour in the studied birds, our results suggest that wing shape should be related with foraging behaviour. It is expected that passerine birds of dense-habitats and ground-dwellers have more rounded wings than species of open-habitats and arboreal-dwellers (Morrison 1982; Yong & Moore 1994; Keast 1996; Amano & Eguchi 2002). Rounded wings allow the needed maneuverability to forage by gleaning in dense habitats (Forstmeier & Keßler 2001; Dawson 2005), which is one of the most important foraging techniques in the leiothrix (in several countries and apparently also in our study area) as well as in the blackcap and robin (Jordano 1982; Cramp & Perrins 1994; Male et al. 1998; Amano & Eguchi 2002; PFP, unpublished observations; Cordier 2002). However, in NE Spain, a foraging experiment conducted in the field found that the leiothrix show a substantial overlap in the foraging niche only with the blackcap (Vall-llosera et al. 2016). Besides, birds with more rounded wings also can improve predator escaping behaviour during ground foraging when compared with species with more pointed wings (Swaddle &

Lockwood 2003). Indeed, a multi-species study revealed that birds with more rounded wings and longer tarsi suffered less predation by sparrowhawks *Accipiter nisus* (Swaddle & Lockwood 1998), which is also the main avian predator in our study area (P. F. P., unpublished observations). As a consequence, the wing shape of the leiothrix is likely to be advantageous for forest dwelling relative to the robin and blackcap, which supports the competition hypothesis.

In summary, the invasive potential of a newcomer may be determined by several traits (including morphological and behavioural), which facilitate moving along different invasion processes. This study adds to the existing body of knowledge on the leiothrix potential as an invasive species (Martin-Albarracin et al. 2015), suggesting mechanisms that may provide competitive superiority and give advantage to the species for establishing populations among native communities. The risks associated with the potential of the leiothrix for invading Europe can be particularly relevant for conservation, particularly, considering its preference for of natural woods, instead of highly disturbed habitats as many other invasive species (e.g. urban areas and farmlands). As discussed by Parker et al. (1999), the degree of impact in the native species must be understood at different scales, from the individual interactions to the processes at the community. Therefore, studies about the competitive superiority of the leiothrix and the effect of its abundance on native species should be conducted in the near future during the first steps of invasion.

Acknowledgements

Appendix 4.1

Results of the first component of principal component analysis of structural body size using wing, bill and tarsus length of small insectivorous woodland passerines. *** $p < 0.001$

Parameter	RV coefficient (Component1)
Wing length	0.806 ***
Bill length	0.922 ***
Tarsus length	0.802 ***
Eigenvalue	2.143
percentage of variance	71.42

Appendix 4.2

Results of the two first components of principal component analysis of exploratory

behaviour of the leiothrix, robin and blackcap. *** $p < 0.001$

Variable	RV coefficient (exploratoryPCA1)	RV coefficient (exploratoryPCA2)
Ground jumps	-0.259	0.959 ***
Branch jumps	0.910 ***	0.004
Flights	0.874 ***	0.2881
Eigenvalue	1.659	0.998
percentage of variance	55.29	33.28

Appendix 4.3

Results of the first component of principal component analysis of wing shape using the

length of ten primaries of the leiothrix, robin and blackcap. ** $p < 0.01$; *** $p < 0.001$

Variable	RV coefficient (wingPCA1)
1st P	0.944 ***
2nd P	0.944 ***
3rd P	0.945 ***
4th P	0.958 ***
5th P	0.924 ***
6th P	0.634 ***
7th P	0.191 **
8th P	-0.230 ***
9th P	-0.517 ***
10th P	0.800 ***
Eigenvalue	5.844
percentage of variance	58.45

Chapter 5 – Dominance of the invasive red-bellied leiothrix (*Leiothrix lutea*) over European native passerine-birds in a feeding context

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Abstract

Behavioural dominance and aggressiveness may be crucial traits facilitating the establishment of an invasive bird species in new environments. However, few experimental studies considered agonistic interactions between exotic and native bird species when they compete for food, particularly when the exotic species is gregarious. Dominance by exotic gregarious species can be particularly important for the conservation of native species because the first often reach very high densities, which can increase their impact over natives. Since sociability can change seasonally and with the time since colonization, it is important to measure heterospecific dominance using pairs of opponents. We aimed to know if individuals of an introduced and invasive passerine species, the red-billed leiothrix *Leiothrix lutea*, are: more aggressive; the initiators of the first interaction; and dominant over native opponents (European robin *Erithacus rubecula*, blackcap *Sylvia atricapilla*) in a feeding context. We performed experiments with pairs of heterospecifics opposing an individual of a native species to an individual of the invasive species. We found that the leiothrix was the initiator of interactions in most experiments, and being apparently dominant, by winning more interactions, over the two native species. However, the invasive species was not more aggressive than the two native species, which suggests that the aggressiveness is not the reason why the invasive species was always dominant. Other possible explanations for its dominance are its larger body size, greater weaponry or specific behavioural traits. The relatively high levels of aggressiveness by the native species indicate they were not indifferent to the invasive species and that it poses a threat to them. This can have negative consequences for the native species, such as an increased risk of injury. Our results suggest that an increase of the leiothrix population may have a negative impact on native species.

Keywords: aggressiveness, competition, *Erithacus rubecula*, exotic, invasive species, *Sylvia atricapilla*

Introduction

Animal populations are mostly regulated by resource availability, density of conspecifics (Fretwell & Calver 1969), and density of heterospecific competitors (Morris 1988). The spreading of an invasive species in a new environment also creates novel interspecific interactions and changes the balance of populations of native species, through competition, predation, hybridization or disease transmission (Martin-Albarracin et al. 2015). Competition for food or space often generates agonistic interactions between individuals. By consistently winning such interactions, some individuals consolidate dominance relationships (Drews 1993) and become more willing to initiate future interactions (Hsu et al. 2006). Invasive species also use aggressive behaviour to attain behavioural dominance over native species, and both if it is an exotic species introduced by humans (Hernández-Brito et al. 2014) or a naturally spreading species (Bennett et al. 2014). Competitive dominance is an expression used in invasion ecology referring to the ability a species has to limit the body condition, reproduction or individual survival of other species (MacDougall & Turkington 2005; Freed & Cann 2009). Ultimately, dominant invasive species can replace the ecological niche of natives leading to their local extinction (Komdeur 1996).

Despite the current interest and relevance of species invasions, few experimental studies considered agonistic interactions between exotic and native bird species when they compete for food (Baker et al. 2014). Recently, Le Louarn et al. (2016), in a study with garden feeders, found evidences of dominance in a feeding context by a gregarious exotic bird species (the ring-necked parakeet *Psittacula krameri*) over a native species. Dominance by gregarious species can be particularly important for the conservation of native species because they often reach very high densities which can increase their impact (Hernández-Brito et al. 2014; Thomson et al. 2015). In feeding contexts,

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gregarious birds may have advantage over non-gregarious birds by learning with conspecifics (Galef & Giraldeau 2001), increasing the displacement success of competitors (Dow 1977; Morgan et al. 2006) and increasing the ratio food intake – time in vigilance (Roberts 1996). Moreover, the sociability of invasive species can vary with time since colonization, as well as, with season (South & Pruett-Jones 2000). Therefore, to evaluate the aggressiveness and behavioural dominance of a gregarious and invasive species it is worthwhile to measure them individually using pairs of opponents. An enclosed-environment study, contrarily to field experiments, allows to exclude the effect of multiple individuals in each aggressive interaction and to eliminate the risk of pseudo-replication with the use of same individuals in different experiments.

Here, we were interested to know if the individuals of an introduced gregarious bird species initiate the interactions more often and are dominant and more aggressive than native opponents in a feeding context using heterospecific pairs of opponents. We selected three potentially competitor passerine birds: (1) the red-billed leiothrix *Leiothrix lutea* (henceforth leiothrix; Family Timaliidae), native from south-eastern Asia, which is considered one of the most harmful avian-invaders (e.g. competition with natives; Martin-Albarracin et al. 2015) and has been introduced in several European countries (Spain: Herrando et al. 2011; Italy: Farina et al. 2013; France: Dubois & Cugnasse 2015; Portugal: Pereira et al. 2017; chapter 4); (2) the European robin *Erithacus rubecula* (henceforth robin; Family Muscicapidae) and (3) the blackcap *Sylvia atricapilla* (Family Sylviidae) which are two European native species. The leiothrix has some characteristics that make it a suitable model to measure competitive dominance over native species: (1) it prefers to invade natural habitats (e.g. dense woods), which may increase their impact over native species (Herrando et al. 2010; Sol

et al. 2012; Farina et al. 2013); (2) it has a similar feeding ecology to the robin and blackcap (e.g. insectivorous-frugivorous diet) and has become established in Europe possibly by having some competitive advantages over them (e.g. wider bill gape; Pereira et al. 2017; chapter 4). The current knowledge on the interactions between the leiothrix and native species is poor and contradictory: there are some evidences of aggressiveness towards native heterospecifics in natural conditions (Conant 1977; Herrando et al. 2011) and also in captivity (Karsten 2006), while an observational field study with feeders suggested that interspecific aggressiveness should be rare (Vall-llosera et al. 2016). The leiothrix has gregarious habits during winter when natural resources are scarcer (Vall-llosera et al. 2016), which can be a confounding factor when we are interested in measuring the behavioural dominance and aggressiveness of each individual. Thus, further studies are needed to determine if the leiothrix is dominant over the native species, a fact that could facilitate its invasive success. We designed a field experiment to test the existence of competition over food in an enclosed artificial environment opposing heterospecific pairs of individuals. Specifically, in each experiment, we analysed the occurrence of aggressiveness, the initiator of the first interaction, and the behavioural dominance between the leiothrix and the two native species. We expect that the leiothrix is competitively dominant over the native species, being also the main initiator of interactions considering its successful invasion on natural habitats (Herrando et al. 2010; Sol et al. 2012; Farina et al. 2013) and its competitive advantages (Pereira et al. 2017; chapter 4). We expect that the leiothrix should be more aggressive than the two native species which should contribute to attain competitive dominance over them.

Methods

Study area

The study was performed in central Portugal (40° 3.162'N, 8° 20.540'W), where an introduced population of leiothrix was discovered in 2007 (Matias 2010). The study area included a small stream valley bordered by dense and diverse vegetation rich in fruit producers (mainly *Rubus* spp., *Vitis vinifera*, *Rubia peregrina* and *Hedera* spp.).

Captures and field enclosure experiment

Field work was performed between November 2014 and February 2015. Birds were captured using mist nets and afterwards ringed with a metal ring with a unique code. We weighed (with a Pesola to the nearest 0.5 g) and sexed the individuals, since the body size and the sex can contribute to the behavioural dominance between individuals (Arnott & Elwood 2009; Peiman & Robinson 2010; Martin & Ghalambor 2014). Sex in robins was determined using genetic markers (by CTM – Centro de Testagem Molecular of the Research Centre in Biodiversity and Genetic Resources – CIBIO). Sex in blackcaps was determined based on crown colour (Svensson 1992), while in leiothrix it was determined by spontaneous sex-specific vocalizations during the experiment (Farina et al. 2013). Birds were submitted to the experiments in the same day they were captured. The testing arena was a field tent (base: 1.40 x 1.40 m, height 2.70 m; Appendix II), which provided visual but not acoustic isolation from the environment. Considering the invasive status of the leiothrix and its spreading over the habitats of the robin and the blackcap (Pereira et al. 2017; chapter 4), we decided to introduce the two opponent individuals in the testing arena in two steps differing in 10 minutes: (1) a native individual was introduced first, and (2) after that period we introduced a

leiothrix. Each experiment started with the introduction of the second bird in the tent and lasted for 10 minutes. We performed two test combinations opposing pairs of heterospecifics: leiothrix versus robin (seven experiments) and leiothrix versus blackcap (nine experiments). We used three artificial wood trees with 1.70 m height and two feeders (one in a tree and other on the ground) provided with two food types: 15 mealworms (total ca. 2 g) and 3 dark-red grapes (ca. 1.5 cm diameter). Since the three species are omnivorous, we used two food types to deal with individual preferences and to motivate their feeding. The behaviour of individuals was recorded using a video camera (HDR-CX220, Sony) fixed to the tent's top in a central position. All individuals were released immediately after the experiments. No birds were injured during handling or experimentation, and no fights were recorded.

Behavioural observations

The videos were analysed using the software OBSERVER 9 (Noldus Information Technology, Wageningen, The Netherlands). We measured three behavioural traits for each opponent: aggressiveness, initiator of the first interaction and dominance. In order to measure the aggressiveness, we used the sum of all aggressive interactions performed by each individual: head forward displays (when a bird remains still with the neck partially extended while pointing the bill towards the opponent; Thompson 1960), and attacks (when a bird makes a fast movement towards the other using the feet or the bill as weapon trying to push or fall on the target). The initiator was the individual that performed the first aggressive behaviour towards the opponent in each experiment (Verbeek et al. 1996; Le Louarn et al. 2016). The winner of each aggressive interaction was the individual that displaced the other from its branch (adapted from Pryke &

Andersson 2003). Preliminary video observations allowed us to verify that not all aggressive interactions lead to displacement of the target. Therefore, we decided to divide each interaction in successful or unsuccessful displacement, considering if the target was displaced or not from its branch, respectively. In order to analyse the dominance between the opponents, we classified the dominant individual in each experiment as the bird that made ≥75% of the successful displacements (adapted from Pryke & Andersson 2003).

Data analysis

We analysed the differences in aggressiveness, initiator individual and dominance between the leiothrix and the two native species with chi-squared tests. The weight differences between the species and the effect of individual sex in the dominance between individuals were analysed with a paired t-test. All analyses were performed with the software R 3.3.0 (R-Core-Team 2016). Statistical significance was set as $p < 0.05$.

Results

Birds ate from the supplied food in 50% of the experiments. Native species were restrained from eating by the leiothrix in five experiments while the reverse did not happen. The leiothrix was the heaviest opponent in all experiments (paired t-test = -14.474, $p < 0.001$), with the average weight of individuals from the three species being (mean ± SD): 24.5 ± 1.8 g for the leiothrix, 17.3 ± 0.6 g for the robin and 17.2 ± 1.0 g for the blackcap. The sex of individuals did not affect the behavioural dominance between opponents (paired t-test: $t = 0.927$, $p = 0.37$).

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The leiothrix was dominant in all experiments opposing blackcaps and robins (Table 5.1). Robins were more aggressive than leiothrix (chi-squared = 17.19, p < 0.001; Fig. 5.1.a; Table 5.1), but were unsuccessful at displacing the exotic opponent (Table 5.1). There were no significant differences in aggressiveness between blackcaps and the leiothrix (chi-squared = 3.31, p = 0.07; Fig. 5.1.b; Table 5.1). But blackcaps showed very low success in displacing the exotic opponents (Table 5.1). The leiothrix was the initiator individual in most experiments (vs robins: chi-squared = 7, p = 0.008; vs blackcaps: chi-squared = 5.4, p = 0.02; Table 5.1).

Table 5.1: Aggressiveness and dominance parameters according to the species: aggressiveness (number of aggressive interactions measured in all experiments), initiator (percentage of experiments in which the species was the initiator individual, i.e. performed the first aggressive behaviour towards the opponent) and dominant individual (percentage of experiments in which the species was the dominant individual, i.e. made ≥75% of the successful displacements within an experiment).

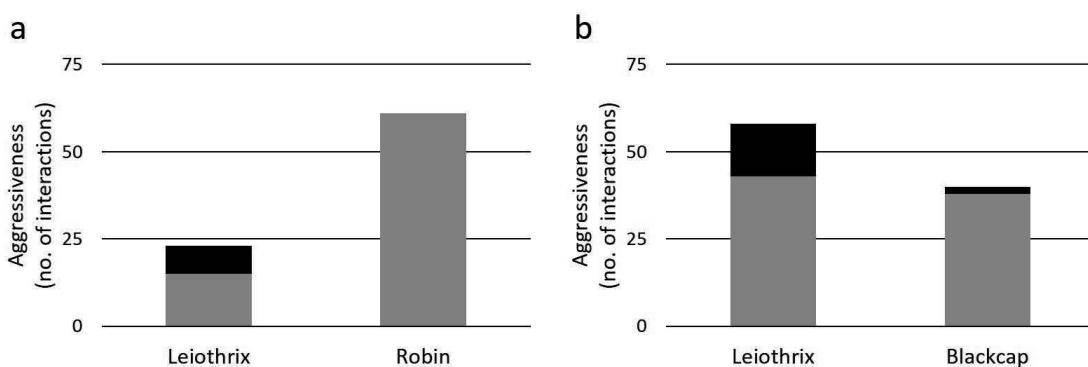| Experiment	Robin Experiments		Blackcap Experiments	
Sample size (no. of experiments)	7		9	
Individual	Leiothrix	Robin	Leiothrix	Blackcap
Aggressiveness (no.)	23	61	58	40
Initiator individual (%)	100	0	89	11
Dominant individual (%)	100	0	100	0

Fig. 5.1: Aggressiveness (number of aggressive interactions measured in all experiments) performed by each species: a) leiothrix versus robin experiments (n = 7); b) leiothrix versus blackcap experiments (n = 9). Bar colours refer to the success of the interaction in displacing the opponent: successful displacements (black) and unsuccessful displacements (grey).

Discussion

Our results indicate that the leiothrix was dominant over native species and was the initiator of the first interaction in most experiments. However, the leiothrix was not more aggressive than the native species, as it showed similar aggression levels as the blackcap and lower than the robin. Therefore, aggressiveness does not seem to be the reason why, in our experiment, the leiothrix was always dominant over the two native species. Alternatively, other possible explanations for its dominance are its larger body size, greater weaponry or other non-agonistic behavioural traits that were not measured in our study (e.g. boldness, exploratory behaviour; Chase & Seitz 2011). Dominance among individuals can be related with exploratory behaviour (Verbeek et al. 1996) or boldness (Sundström et al. 2004). However, the leiothrix and the two native species do not seem to differ in frequency of exploratory behaviours (Pereira et al. 2017; chapter 4), making it unlikely that a greater exploratory capability is an explanation for the dominance shown by the leiothrix. We have no data regarding boldness among the three species which leaves this possibility open.

According to our behavioural observations, all the three species used the same weaponry to attack (bill or feet). Nevertheless, the leiothrix has a larger bill than the two native species (Pereira et al. 2017; chapter 4), which may be advantageous. Moreover, by being heavier, the leiothrix can benefit from further superiority when attacking the two lighter native species, namely by possessing stronger leg musculature (as in other groups of birds: Martin & Ghalambor 2014). Therefore, the weaponry size of the study species does not appear to be independent on their body size. Apparently, the leiothrix may need relatively few interactions to displace native opponents. Considering the similar use of weaponry among the three species, we expected that individuals could be able to assess the fighting ability of their opponents just by visual inspection (Arnott &

Elwood 2009; Peiman & Robinson 2010). Because size is often used to assess fighting ability (Hsu et al. 2006; Arnott & Elwood 2009), this could may benefited the leiothrix, which is the largest of the three species and the initiator in most of our experiments. Interestingly, native species were aggressive towards the larger leiothrix instead of avoiding it. In most interactions, this aggressiveness by the native species was noneffective, as it did not lead to the displacement of the invasive species, and therefore it was insufficient to achieve dominance over the leiothrix. However, considering the exotic status of the leiothrix, these results should be interpreted with caution. For example, a first possible explanation for the high aggressiveness towards the leiothrix is that both native species were not able to anticipate the dominance of the exotic species because individuals of the native species have a reduced or no previous experience with the invasive species (Hsu et al. 2006). A second possible explanation considers that the native species may have recognized the leiothrix as a competitor, and deliberately tried to mob the dominant species similarly to what occurs among raptors of different trophic levels (Lourenço et al. 2011). Considering that natives were not the initiator of the first interaction in most experiments, the first explanation appears to be more probable to justify the high aggressiveness of natives.

To the best of our knowledge, this is the first experimental work in which clear dominance by an exotic bird species over native rivals was confirmed in a feeding context using pairs of opponents. Although the results were obtained in a controlled environment, it is likely that the interactions among the involved species in nature are similar. Our work is also original considering the difference between levels of aggressiveness and dominance outcomes. Subordinate species are likely to avoid dominant species and reduce the signals that induce aggressiveness from dominant opponents (Martin et al. 2017). However, in our study, the native species showed high

levels of aggressiveness towards the invader which may have several negative consequences for them such as an increased risk of injury (Parker 1974) or exposure to predators (Lange & Leimar 2001). The dominance of an invader over smaller native species can reduce the availability of resources for the latter and ultimately contribute to reduce their populations, as occurred with the invasion of the noisy miner *Manorina melanocephala* in Eastern Australia (Thomson et al. 2015). Currently, the robin and the blackcap co-occur with the leiothrix in different European countries and population declines of native species have been recorded in some invaded regions (Basly 2007; Herrando et al. 2010; Farina et al. 2013; Pereira et al. 2017; chapter 4). Our results suggest that a possible future outcome is a considerable limitation of native species populations by the leiothrix if the population of the exotic species maintains an increasing trend. The impact of the leiothrix on native populations can be stronger than expected taking into account its gregarious habits, which can contribute to numerical dominance in interspecific interactions. We highlight the relevance of studying exotic species during the first establishment periods in order to prevent or reduce their negative impacts on native biodiversity.

Acknowledgements

Chapter 6 – Evidence for interspecific competition through singing behaviour between distantly related passerine birds

This chapter will be submitted as research article to the "**Animal Behaviour**" journal

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Abstract

Interspecific competition is rarely assessed between distantly related species, although they can compete for some ecological resources. Here we wanted to assess the occurrence of interspecific aggression resulting from direct competition between two distantly related passerine species (henceforward focal species), the robin (*Erithacus rubecula*) and the blackcap (*Sylvia atricapilla*), that are potential competitors for habitat and feeding resources. In order to make the comparison more robust, we considered two other species that are either unlikely or new competitors. Thus, we also considered the great tit (*Parus major*) as unlikely competitor, and the exotic red-billed leiothrix (*Leiothrix lutea*), which competes with the robin and the blackcap for ecological niche and is dominant over them. We used interactive playbacks to measure individuals' singing behaviour responses towards challenges from conspecifics and heterospecifics. In general, robins and blackcaps showed a stronger response towards conspecifics than against all heterospecifics. While in some parameters, robins presented no differences against conspecifics and native competitors. On the contrary, blackcaps were affected by the robin playback suggesting an asymmetric response between the two species when consider the native competitor. We discussed these differences between native competitors and their motivation to incur in graded disputes considering their ecological requirements. The responses of the focal species did not differ between the exotic competitors and non-competitors or were weaker in relation to the exotic competitor. These results suggest that the exotic competitor was not recognized as a rival but the focal species may also have some reluctance to sing after the exotic species. Our study suggests that passerines can use the singing behavioural as an aggressive signal towards distantly related species. We highlight the importance of this kind of studies to evaluate the effect of coexistence-time on the behavioral response among native and exotic competitor species.

Keywords: coexistence, interspecific aggressiveness, overlapping playback, territorial intrusion, bird song

Introduction

Interspecific competition is considered an important factor affecting the distribution of species and the fitness of individuals, but is often only assessed between closely related species, although there are no reasons to exclude the possibility of it occurring between more distantly related species (Schoener 1983; Pigot & Tobias 2013). Also, the co-occurrence is not a proof of the absence of competition and evolved compatibility, since overlapping interests will cause some sort of conflict (Amarasekare 2003). Territorial behaviour is one of the most common ways animals use to mediate conflicts for resources and warrant exclusive access (Maher & Lott 1995). Territoriality can also exist at the inter-specific level, between species with similar ecological niches (Cody 1978; Losin et al. 2016) and, in these cases individuals behave aggressively towards both conspecifics and heterospecifics (Walls 1990; Anderson & Grether 2010). Studies in interspecific aggression show that its intensity tends to decrease with increasing territory overlap (birds: Robinson & Terborgh 1995; fishes: Genner et al. 1999; insects: Tanner & Adler 2009).

Cases of aggression between distantly related species of passerine birds have been reported in nature and appear to be widespread considering the variety of taxonomic families studied: between Emberizidae and Troglodytidae (Gorton 1977), Fringillidae and Paridae (Reed 1982), Icteridae and Troglodytidae (Bump 1986), Parulidae and Tyrannidae (Martin et al. 1996). These studies used the song as mechanism to measure the interspecific aggressiveness. However, only Martin et al. (1996) discussed the relationship between the interspecific aggressiveness and competition, which highlights the necessity of additional research on aggressive behaviour between distantly related species.

The introduction of exotic species in a new environment often generates conflicting interactions with native species and contributes to new forms of interspecific competition. These introductions also provide opportunities for testing how native species react to newly arrived potential competitors, and respond to non-coevolved aggressive signals, since they often compete aggressively for resources (Holway & Suarez 1999; Tanner & Adler 2009; Chapple et al. 2012; Hudina & Hock 2014). However, the existence of territorial behaviour between heterospecifics in in bird communities invaded by bird species is not yet well understood because most studies focused (1) on the perspective of the exotic species as the aggressor, which can vary in intensity according to its population size, and (2) on the disputes for small areas (e.g. tree holes) rather than for the whole territory space (Lowe et al. 2011; Grundy et al. 2014; Hernández-Brito et al. 2014).

Birdsong is generally used for mate attraction and territory defence and can be used signal an agonistic intent (Searcy & Beecher 2009; Funghi et al. 2015a). Vocal interactions between territorial birds can arise within their own territorial boundaries or during a territorial intrusion (Naguib 2005), where the territorial owner can show great aggressiveness by approaching (or standing nearby) its opponents or decreasing the latency to sing after them (Briefer et al. 2008; Freeman 2016; Gordinho et al. 2016); or, through the exhibition of songs with broad frequency bandwidth or low peak frequency (DuBois et al. 2008; de Kort et al. 2009; Linhart et al. 2012). The simulated territorial intrusion using song playbacks is a widely used experimental technique to measure the aggressive response of territorial bird species (Searcy & Beecher 2009). A playback which overlaps the song of a territorial bird (termed overlapping playback) is a way to motivate an aggressive response of the territory owner because song overlap is an

aggressive signal among birds (Dabelsteen et al. 1997). However, territorial intrusions using song playbacks of heterospecifics has been mostly applied between species within the same-family rather than between distantly related species (e.g. Robinson & Terborgh 1995; Matyjasiak 2005; Reif et al. 2015; Freeman 2016).

In the Northern Hemisphere, communities of insectivorous woodland passerines are composed by several songbird species with territorial habits which may present several levels of interspecific competition. The fact that much is known on their behaviour and ecology makes them suitable to study interspecific competition and aggressiveness (Cody 1978; Martin et al. 1996; Hansen & Slagsvold 2003; Matyjasiak 2005; Reif et al. 2015; Losin et al. 2016). Here we wanted to assess the occurrence of interspecific aggression resulting from direct competition between two distantly related passerine species (henceforward focal species), the robin (*Erithacus rubecula*; Family Muscicapidae) and the blackcap (*Sylvia atricapilla*; F. Sylviidae), that occur sympatrically in most of Europe and are potential competitors for habitat and feeding resources (Leach 1981; Cramp & Perrins 1994; Hernández 2008; chapters 2 and 3). In order to make the comparison more robust, we considered two other species that are either unlikely or new competitors. Thus, we also considered the great tit (*Parus major*; F. Paridae) as unlikely competitor, and the exotic red-billed leiothrix (henceforward leiothrix; *Leiothrix lutea*; F. Timaliidae) newly introduced from Asia as a new competitor, which competes with the robin and the blackcap for ecological niche and is dominant over them (Vall-llosera et al. 2016; Pereira et al. 2017; chapter 4). All four species are small insectivorous passerines which can be considered vocally dominant species (regarding the number of songs per minute) in European woodland communities (Farina et al. 2013; 2015). Robins and blackcaps have relatively small breeding

territories, rich vocal repertoires and fast aggressive response to territorial intrusions from conspecifics (Cramp & Perrins 1994; Dabelsteen et al. 1997; Matyjasiak 2005). This study was designed to determine if individuals responded aggressively towards interspecific competitors and if by their responses we could determine whether they identified the challenge of a territorial trespasser from a particular species as threatening. We measured the territorial overlap between individuals from different species and used interactive playbacks to measure individuals' singing behaviour responses towards challenges from conspecifics and heterospecifics.

Territoriality results from the competition for resources, and aggression is a commonly used behaviour for territory defence (Krebs & Davies 2009). Therefore, based on the ecological similarities among these species we expect to find: (1) greater territory overlap between focal species and non-competitors than with competitors; and (2) greater aggressiveness towards conspecifics than towards heterospecifics. Additionally, if the focal species uses the song to signal aggressiveness to heterospecifics, then we expect that: (3) the response towards native competitors should be more aggressive than towards non-competitors; (4) the response towards exotic competitors should be less aggressive than towards non-competitors, indicating that the focal species is subordinate to the exotic species (Robinson & Terborgh 1995).

Methods

Study area, territory measurements and song recording
The study was performed in central Portugal (40° 3.162'N, 8° 20.540'W) in a small stream valley bordered by dense and diverse oak-woodland (*Quercus robur*) rich in scrubs (Appendix IV).

The field work was performed during the breeding season of robins and blackcaps, from May to June 2015. The territories of robins and blackcaps were identified by performing several linear transects in the study area. In each territory, we monitored the singing perches for at least three consecutive days and used this information to determine and plot the territorial boundaries. The two other species (the great tit and the leiothrix) were also monitored, to estimate territorial overlap among the four study species. Focal individuals had at least one neighbour of the native competitor species and the mean overlapping territorial area with them was 58 ± 14 % (mean ± standard error) for the focal robins and 44 ± 11 % for the focal blackcaps. The territories of the exotic competitor and the non-competitor showed a 100 % overlap with the focal individuals. However, they were not neighbours in all cases: exotic competitors were neighbours to 12 focal robins and to 13 focal blackcaps; whereas non-competitors were neighbours to 11 focal robins and to 15 focal blackcaps. No focal individual presented overlapping territories with conspecifics.

We generated playback stimuli from high-quality recordings without overlapping vocalizations of other individuals that were collected in different regions of the country to reduce the probability of familiarization with the focal individuals. A band-pass filter was used to remove the background noise under 1000 Hz and above 10000 Hz and subsequently normalized to 90% of the maximum volume in Avisoft-SASLab Pro (Version 5.2.06, R.Specht, Glienicke, Germany). Each stimulus was composed by a song recorded from different individuals, and we used a total of 36 different stimuli from the four study species (10 robins, 10 blackcaps, 8 great tits and 8 leiothrix).

We tested 32 individuals with known territorial boundaries (16 robins and 16 blackcaps). Experiments were performed between 6:00 am and 1:00 pm near the

territory centre of the focal individual. The singing behaviour of the tested birds was recorded with a directional long shotgun microphone (Sennheiser MKH 70 P48, Wedermark-Wennenbostel, Germany) and into a portable digital recorder (Marantz Professional PMD661 MKII, Kanagawa, Japan). The tests included a period of recording of spontaneous singing (5 minutes), followed by the playback stimulus that was used to overlap the focal bird song (3 minutes) and a refractory period where the bird was recorded (5 minutes). The birds were subject to four playback stimuli presented in the same day, one for each condition: conspecific, native competitor (robin/blackcap), native non-competitor (great tit) and exotic competitor (leiothrix). The minimum interval between tests with different playbacks was 15 minutes, with the order of presentation being randomized.

We used the playback of a song and a dummy perched in an artificial tree with 1.7 m high to simulate the territorial intrusions for each condition (Appendix IV). A blinded-selected stimulus was played by overlapping the songs of focal individual during three minutes from a Sony SRS-A 57 loudspeaker at a peak volume of 80 dB measured 1 m from the speaker with a Tenma RS-232 72-860A Sound Level Meter (Tenma, Taipei, Taiwan). Each stimulus was used on average in 1.8 ± 0.3 experiments (mean $\pm$ standard error) by each focal species. During the experiment, the observer remained concealed in the vegetation about 10 m away from the speaker. We changed the dummy between each playback stimulus. If the focal bird became silent after the playback presentation, we extended the recording period until the first song was produced in order to measure the latency to sing after the playback. For each recorded song, we estimated the distance (to the nearest metre) between the singing perch and the loudspeaker to measure the

minimum distance during the playback and the time spent singing within 10 m after the playback. There were no attacks to the dummies by focal individuals in any experiment.

Behaviour and sound analysis

Three behavioural variables of the focal individual were measured in each test: (1) *minimum distance* to the speaker (in metres) during the 3 minutes of playback; (2) *latency to sing*, as the time until the first song of the focal individual after the end of the playback measured in seconds; and (3) *time within 10 m* (in seconds), as the time spent singing by the focal individual within 10 m to the speaker during the 5 minutes following the playback. The singing behaviour of the focal bird was analysed using Avisoft SASLab Pro. We measured acoustic parameters in three randomly selected songs from the spontaneous recording and the same number of songs from the 5 minutes after each playback. The parameters were: (1) *frequency bandwidth* of the song, as the difference between the maximum and the minimum frequency of the song in kHz; (2) *peak frequency*, as the frequency at the point of maximum amplitude of the songs in kHz.

Beyond the function as aggressive signal, song overlapping can be a source of disruption, particularly when individuals compete for acoustic space (Brumm 2006; Malavasi & Farina 2013). In order to exclude the effect of the acoustic competition in the response of the focal bird, we measured and compared the acoustic parameters of the stimulus and the spontaneous recordings, for which we considered three frequency variables – maximum, minimum and peak frequency –, and song duration in seconds. The analysed songs did not show any temporal overlap with other vocalizations.

Data analysis

All variables were analysed to detect outliers and check distribution. For the analyses of acoustic space similarities among species, we performed a principal component analysis (PCA) on the four acoustic variables: maximum frequency, minimum frequency, peak frequency and song duration, collected from 96 spontaneous songs of the focal birds (two species) and 36 song stimuli (four conditions). The centroids of the song groups (two focal species and four conditions) in the PCA were used to measure the Euclidean distance between them, which is considered a suitable measure of song similarity (Wheatcroft & Price 2013). The first component of PCA describing song similarity explained 43.31% of the overall variation and had a strong ($p < 0.001$) and positive correlation with the three frequency parameters, but was negatively correlated with the song duration (Appendix 6.1). The Euclidean distances between the spontaneous songs of focal birds and the stimuli showed, as expected, higher similarity with the conspecific stimuli (Appendix 6.2). Considering the heterospecifics, focal robins showed higher similarity with the great tit stimuli and focal blackcaps showed higher similarity with the robin stimuli.

The effect of species-type playback (that is: conspecific, native competitor, exotic competitor and non-competitor) on the behavioural response of each of the focal species was tested using three generalized linear mixed models with Poisson distribution, for three of the dependent variables: minimum distance, latency to sing and time within 10 m, and using individual identity as a random factor. For the acoustic response of focal birds (frequency bandwidth and peak frequency) we used linear mixed models with normal distribution, and individual identity as a random factor. For the acoustic variables, and in order to measure individual response, we calculated and used the difference between the average of three songs after the playback minus the average of

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the subject's spontaneous songs. We performed an Anova type II after each linear mixed model to obtain p values for the analyses of the acoustic parameters.

The comparisons between species-type playbacks were done by pairwise comparisons with post hoc Tukey-tests. All analyses were performed using the software R 3.3.0 (R-Core-Team 2016) with the packages lme4 (Bates et al. 2014), car (Fox et al. 2016), lsmeans (Lenth 2013) and ggplots2 (Wickham 2016).

Results

The individuals from both focal species revealed general differences in their response in relation to the condition, for the variables – minimum distance, latency to sing, time within 10 m and frequency bandwidth (Tables 6.1, 6.2). The peak frequency of the focal individuals did not show a significant variation between conditions (Table 6.2).

Response towards conspecifics versus all heterospecifics

Robins sang at a closer distance (*minimum distance*) and spent more *time within 10 m* against playbacks from conspecifics than against all heterospecifics (Fig. 6.1.a and 6.1.c; Table 6.3) but showed no differences in the *latency to sing* against conspecifics and native competitors with very low values for both types of opponents (z = - 1.734, p = 0.306). There were no differences in the *frequency bandwidth* against conspecifics and native competitors (z = 1.510, p = 0.431), as well as against exotic competitors (z = 1.697, p = 0.325).

For blackcaps there were no significant differences in *minimum distance* when comparing conspecifics with all other species, but they showed a shorter *latency to sing*

and spent more *time within 10 m* against conspecific playbacks than against heterospecifics (Fig. 6.1; Table 6.3). There were no differences in *frequency bandwidth* when comparing conspecifics with exotic competitors (z = 0.523, p = 0.954) and non-competitors (z = 0.867, p = 0.822).

Table 6.1: Statistical parameters of the generalized linear mixed models measuring the effect of the condition in the parameters of the singing behaviours measured on the focal species: "minimum distance during the playback", "latency to sing" and "time within 10 m".

Focal species: analyses	species treatments	Estimate	Std. Error	z-value	P
Robin: minimum distance	intercept	2.21390	0.09011	24.570	<0.001
	native competitor	0.39186	0.10654	3.678	<0.001
	exotic competitor	0.41443	0.10606	3.908	<0.001
	non-competitor	0.39186	0.10654	3.678	<0.001
Robin: latency to sing	intercept	0.03073	0.28968	0.106	0.916
	native competitor	0.31482	0.18159	1.734	0.083
	exotic competitor	3.04318	0.14132	21.534	<0.001
	non-competitor	2.81648	0.14214	19.815	<0.001
Robin: time within 10 m	intercept	3.20003	0.25981	12.32	<0.001
	native competitor	-1.85385	0.09373	-19.78	<0.001
	exotic competitor	-1.62921	0.08519	-19.12	<0.001
	non-competitor	-1.41636	0.07807	-18.14	<0.001
Blackcap: minimum distance	intercept	1.97804	0.09492	20.840	<0.001
	native competitor	0.50391	0.11771	4.281	<0.001
	exotic competitor	0.46677	0.11854	3.937	<0.001
	non-competitor	0.42256	0.11957	3.534	<0.001
Blackcap: latency to sing	intercept	1.86291	0.26207	7.108	<0.001
	native competitor	1.06361	0.08269	12.863	<0.001
	exotic competitor	1.03646	0.08298	12.491	<0.001
	non-competitor	1.03796	0.08296	12.511	<0.001
Blackcap: time within 10 m	intercept	2.91640	0.25902	11.259	<0.001
	native competitor	-1.70149	0.10009	-16.999	<0.001
	exotic competitor	-0.46689	0.06333	-7.372	<0.001
	non-competitor	-0.27897	0.05990	-4.657	<0.001

Table 6.2: Statistical parameters of the Anova type II measuring the effect of the condition in the acoustic parameters.

Focal species: analyses	Chisquare	Df	P
Robin: frequency bandwidth	18.701	3	<0.001
Robin: peak frequency	5.1652	3	0.160
Blackcap: frequency bandwidth	12.969	3	0.005
Blackcap: peak frequency	5.7348	3	0.125

Response towards native competitors versus non-competitors

Robins had a considerably shorter *latency to sing* against playbacks of native competitors than against non-competitors ($z = -20.389$; $p < 0.001$; Fig. 6.1.b) and it was similar to the response to conspecifics. Also, robins showed a higher different, and broader *frequency bandwidth* against native competitors than against non-competitors ($z = 2.88$, $p = 0.021$). However, there were no differences in *minimum distance* between the response towards playbacks of native competitors and non-competitors ($z = -0.238$; $p = 0.995$), while robins also spent less *time within 10 m* near playbacks of native competitors than non-competitors ($z = -3.913$; $p = 0.001$). Blackcaps did not show variation in *minimum distance* and *latency to sing* between native competitors' playbacks and non-competitors (Fig. 6.1.a and 6.1.b; Table 6.3), but spent less *time within 10 m* against native competitors than non-competitors ($z = -13.872$; $p < 0.001$; Fig. 6.1.c), as if avoiding the first.

Response towards exotic competitors versus non-competitors

The responses of the focal species did not differ between the exotic competitors and non-competitors or were weaker in relation to the exotic (Fig. 6.1; Table 6.3). Robins showed a shorter *latency to sing* against exotic competitors' playbacks ($z = 5.008$; $p < 0.001$; Fig. 6.1), while blackcaps spent less *time within 10 m* against the playbacks of non-competitors than of the exotic competitors ($z = -2.799$; $p = 0.026$; Fig. 6.1.c).

Table 6.3: Pairwise comparisons between conditions for each behavioural parameter measured on the focal species.

| Focal species: behavioural parameter	Conspecific - Native Competitor	Conspecific - Exotic Competitor	Conspecific - Non-competitor
Robin: minimum distance	z = -3.362; p = 0.004	z = -3.958; p < 0.001	z = -3.594; p = 0.002
Robin: latency to sing	z = - 1.734; p = 0.306	z = -21.534; p < 0.001	z = -19.815; p < 0.001
Robin: time within 10 m	z = 19.78; p < 0.001	z = 19.124; p < 0.001	z = 18:143; p < 0.001
Robin: frequency bandwidth	z = 1.510; p = 0.431	z = 1.697; p = 0.325	z = 4.289; p < 0.001
Robin: peak frequency	z = 0.012; p = 1	z = -1.240; p = 0.6	z = -1.854; p = 0.248
Blackcap: minimum distance	z = -2.520; p = 0.057	z = -2.469; p = 0.065	z = -0.978; p = 0.762
Blackcap: latency to sing	z = -12.863; p < 0.001	z = -12.490; p < 0.001	z = -12.511; p < 0.001
Blackcap: time within 10 m	z = 16.999; p < 0.001	z = 7.372; p < 0.001	z = 4.657; p < 0.001
Blackcap: frequency bandwidth	z = 3.372; p = 0.004	z = 0.523; p = 0.954	z = 0.867; p = 0.822
Blackcap: peak frequency	z = 1.796; p = 0.275	z = 1.268; p = 0.583	z = -0.275; p = 0.993
	Native Competitor - Exotic Competitor	**Native Competitor - Non-competitor**	**Exotic Competitor - Non-competitor**
Robin: minimum distance	z = -0.614; p = 0.928	z = -0.238; p = 0.995	z = 0.376; p = 0.982
Robin: latency to sing	z = -22.410; p < 0.001	z = -20.389; p < 0.001	z = 5.008; p < 0.001
Robin: time within 10 m	z = 1.922; p = 0.219	z = -3.913; p = 0.001	z = -2.032; p = 0.176
Robin: frequency bandwidth	z = 0.289; p = 0.992	z = 2.88; p = 0.021	z = 2.453; p = 0.068
Robin: peak frequency	z = -1.261; p = 0.588	z = -1.874; p = 0.239	z = -0.582; p = 0.938
Blackcap: minimum distance	z = 0.517; p = 0.999	z = 1.553; p = 0.406	z = 1.501; p = 0.437
Blackcap: latency to sing	z = 0.455; p = 0.969	z = 0.43; p = 0.973	z = -0.025; p = 0.999
Blackcap: time within 10 m	z = -11.805; p < 0.001	z = -13.872; p < 0.001	z = -2.799; p = 0.026
Blackcap: frequency bandwidth	z = -2.802; p = 0.026	z = -2.473; p = 0.064	z = 0.336; p = 0.987
Blackcap: peak frequency	z = -0.545; p = 0.948	z = -2.023; p = 0.180	z = -1.512; p = 0.431
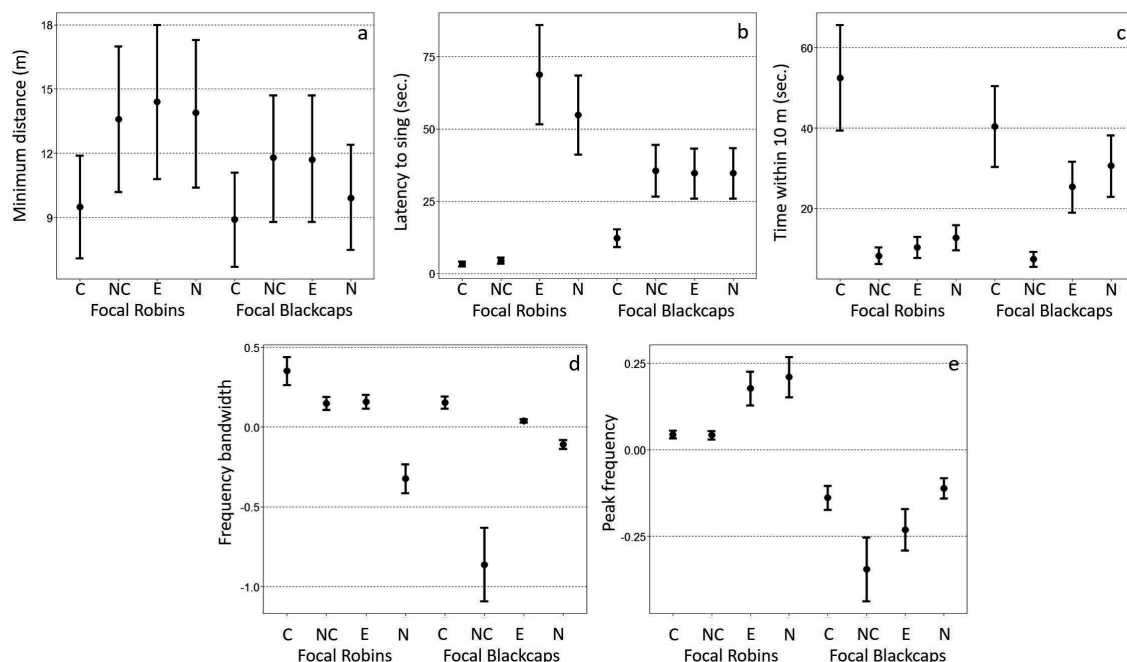
Fig. 6.1: Mean and error bars of the behavioural parameters (minimum distance, latency to sing and time within 10 m) and acoustic parameters (frequency bandwidth and peak frequency) measured from the focal birds according the condition: spontaneous song (S); and after the playback song of four species, which were (C) a conspecific, a native competitor (NC; a blackcap or a robin playback song, depending on the focal species), red-billed leiothrix as an exotic competitor (E) and a great tit as non-competitor (N). For each acoustic parameter, we subtracted the value measured before the playback to the value measured after the playback (i.e. positive values indicate an increase in the parameter value after the playback).

Discussion

The behavioural and acoustic parameters of focal individuals were affected by the playback of conspecifics and, in some cases, also by the playback of heterospecifics. The responses of focal individuals could have an aggressive intent or be dependent on the competition for acoustic space. In general, the intensity of responses was stronger towards conspecifics and weaker towards heterospecifics with greater territory overlap which suggests an aggressive intention, such as observed in several bird taxa (Cody 1978; Robinson & Terborgh 1995; Pyke et al. 1996; Losin et al. 2016). However, considering that the stimuli were played by overlapping the songs of the focal individuals, their responses could also reflect a response to competition for acoustic

space (Brumm 2006; Malavasi & Farina 2013). Masking song frequencies affects signal broadcasting, ultimately leading individuals to change their positions or singing periods (Naguib 2005). Accordingly, in our study if the playbacks affected the acoustic performance of focal individuals, then we would expect that birds stayed away from the loudspeaker during the playback and/or started to sing just after its finish (McLaughlin & Kunc 2013; Farina & Pieretti 2014). This behavioural response was observed in the focal robins after the blackcap playback (a competitor). The absence of a reverse response by the blackcap (relatively to the robin playback) could be related with the singing behaviour of species, since the robin sings mainly during the refractory period of other species instead of jamming their songs with others such as the blackcap (Malavasi & Farina 2013). This suggests that the time availability is more limiting for singing robins than for singing blackcaps. If the response of focal robins to the blackcap playback was only dependent on acoustic competition, we would expect a similar response to the playback of the great tit – a non-competitor species which has higher song similarity with robin songs than blackcap songs do. However, focal robins showed a much shorter latency to sing after blackcaps than after great tit, which supports the existence of an aggressive reaction of robins to the blackcap song.

Considering that the aggressive behaviour is costly, individuals should be selective on their rivals according to their familiarity (Briefer et al. 2008) or genetic relativeness (Gordinho et al. 2016). The encounter probability between individuals (and the consequent familiarity between them) can be an important step for the identification of a rival, increasing with species densities, habitat similarities, and temporal habits (Grether et al. 2009; Ord et al. 2011). During such encounters individuals can assess the fighting ability of the opponents even without incurring in aggressive contests (e.g. evaluation of weaponry; Arnott & Elwood 2008; Számadó 2008). The use of the same foraging

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habitats can increase the encounter probability between individuals (Hansen & Slagsvold 2003; Matyjasiak 2005; Losin et al. 2016). Therefore, during the breeding season, the singularity of foraging habitat of robins among the studied species (a ground forager among three arboreal foragers; Cramp & Perrins 1994; Adamík et al. 2003; Vall-llosera et al. 2016), may reduce the encounters between robins and other species and thus reducing the occurrence of aggressive interactions between them. However, interspecific interactions during the remaining part of the year can have also an important role in heterospecific recognition because robins and blackcaps compete aggressively for fruits outside the breeding season (Leach 1981; Hernández 2008). Generally, long-term coevolution plays a more important role in rival recognition than the short-term experience obtained along an individual's life span (Le Breton et al. 2007; Grether et al. 2009). Therefore, the fact of not sharing a period of coevolution should have an important effect in the responses of our focal species towards the exotic competitor as the familiarity between them is relatively low. For most behavioural parameters, focal birds showed a response to the leiothrix similar to the non-competitor species (although long-term coevolved species), the great tit. This result can indicate that the leiothrix was not recognized as a rival by the focal individuals. However, after the playback of the exotic competitor, focal robins increased their latency to sing and focal blackcaps spent few time near the loudspeaker. These behaviours are generally considered as revealing a subordinate status (Robinson & Terborgh 1995; Martin et al. 1996; Freeman 2016), suggesting that the two focal species may have some reluctance to sing after the exotic species.

The aggressive behaviour among heterospecific individuals can also reflect an overlap in the resources used (Peiman & Robinson 2010). The intensity of responses to heterospecifics measured in our study can be related with different degrees of

competition for breeding sites between species, as robins and blackcaps compete for singing perches and nest in densely covered sites below the tree layer, while the great tit nests in tree-holes (Cramp & Perrins 1994). However, when considering only the responses between robins and blackcaps, their intensity was asymmetric: the robin showed a stronger response towards the blackcap playback than the reverse. In a recent study, Martin and colleagues (2017) found that the aggressive behaviour among closely related species is generally asymmetric. The responses we measured between the robin and the blackcap suggest that asymmetric aggressiveness can also occur between distantly related species. This pair of species competes for nesting habitat, however, the robin needs more limiting resources (higher vertical development of vegetation) than the blackcap (chapter 2). Since the motivational state of individuals can increase their aggressiveness behaviour, particularly when competing for limiting resources (Gabor & Jaeger 1995; Arnott & Elwood 2008), the asymmetric response measured between robins and blackcaps could have an aggressive purpose.

Our study suggests that passerines can use the singing behavioural as an aggressive signal towards distantly related species. Interspecific competition for habitat can modulate the degree of territorial overlap between the four species. The asymmetric response observed between our focal species may be related with different degrees of specialization in their nesting habitat. Also, our results showed that the exotic competitor species did not evoke more aggressiveness than the non-competitor species, which can indicate that the focal individuals did not identify it as a rival. However, some behavioural parameters measured in the focal species suggest a subordinate response towards the exotic competitor. Similar approaches to our work but in other study areas with different times of colonization by exotic species may help us evaluate

the effect of coexistence-time on the behavioural response among native and exotic competitor species.

Acknowledgements

Appendix 6.1

Results of the two first components of principal component analysis of the acoustic parameters of the spontaneous song of focal individuals and the song playbacks using the minimum frequency, maximum frequency, peak frequency and song duration. ** $p < 0.01$; *** $p < 0.001$.

Variable	RV Coefficient (component 1)	RV Coefficient (component 2)
minimum frequency	0.817 ***	-0.323 ***
maximum frequency	0.632 ***	0.602 ***
peak frequency	0.753 ***	0.213 **
Duration	-0.315 ***	0.879 ***
Eigenvalue	1.732	1.285
Percentage of Variance	43.31	32.12

Appendix 6.2

Euclidean distance (ED) between the spontaneous songs of the focal individuals and the four species playbacks using the minimum frequency, maximum frequency, peak frequency and duration of songs.

Song type	ED to Focal Robin	ED to Focal Blackcap	sample size	minimum frequency (Hz)	maximum frequency (Hz)	maximum peak frequency (Hz)	duration (sec.)
Focal Blackcap	-	-	48	1794 ± 259	7819 ± 1129	3544 ± 512	3.6 ± 0.5
Focal Robin	-	-	48	2556 ± 369	8865 ± 1280	3798 ± 548	2.1 ± 0.3
Playback Blackcap	2.26	1.88	10	1870 ± 591	5850 ± 1850	3390 ± 1072	1.9 ± 0.6
Playback Great Tit	1.16	2.26	8	2763 ± 977	6225 ± 2201	3988 ± 1410	2.3 ± 0.8
Playback Leiothrix	3.96	2.82	8	1363 ± 482	3975 ± 1405	2788 ± 986	2.6 ± 0.9
Playback Robin	0.91	1.91	10	2400 ± 759	8750 ± 2767	4250 ± 1344	2.6 ± 0.8

Chapter 7 – General Discussion

In this thesis, I combined information on micro-habitat preferences, eco-morphological traits and two behavioural complexes (foraging and singing behaviour) to found evidence of interspecific competition and to explore the mechanisms that allow the coexistence of distantly related birds that are potential competitors. Three species were used as models: the European robin, the blackcap and the red-billed leiothrix, which revealed to be suitable for these purposes of the thesis. In synthesis, I found complementary evidences of interspecific competition among them, such as (1) the occurrence of habitat shift between the robin and the blackcap, (2) relatively high levels of heterospecific aggressiveness between the robin and the blackcap while competing for food (3) a high overlap of the morphological niche between the leiothrix and (mainly) the robin, (4) behavioural dominance of the leiothrix over native species in feeding context, and (5) the use of singing behaviour by the robin and the blackcap as an aggressiveness signal to heterospecifics.

Species with similar ecology can coexist when they diverge in such traits that will reduce competition intensity (Leal & Fleishman 2002; Krishnan & Tamma 2016). In chapter 2, I analysed how the co-occurrence with heterospecifics affects the habitat used by the robin and the blackcap. These two species present some characteristics compatible with the development of ecological shifting, such as wide geographical distributions and broad ecological niches (Connell 1980; Case et al. 2005; Davies et al. 2007). Moreover, they occur in syntopic and allotopic conditions which allow to analyse the existence of ecological shifting between them (Rivas 1964; Beaver & Baldwin 1975; Jenssen et al. 1984; Lisičić et al. 2012). My results indicated that at a landscape-scale (the broad scale), robin and blackcap were influenced by the same

characteristics, with both species being more abundant in native broadleaf woods. However, at the site-scale – a smaller scale than landscape, in which the individuals interact directly and compete for resources (Wiens 1989; Huston 1999; Kneitel & Chase 2004) – the habitat used by the robin and the blackcap differed only when in syntopy: sites used by robins had higher number of vegetation layers than those used by blackcaps, whereas the latter used sites with denser scrub cover. These differences in habitat can be related with their feeding and nesting habits. For example, the vertical stratification of woody vegetation can reduce insolation and soil drought, which in turn increases the abundance of ground invertebrates for the robin (Ludvig et al. 1995; Sánchez et al. 2010; Tellería 2015). However, in the absence of the respective heterospecific, both species used sites with similar characteristics of woody vegetation, including similar number of vegetation layers and scrub cover, indicating the existence of habitat shifting between them when in syntopy. In birds, the research on habitat shifting of distantly related species is scarce (Landres & MacMahon 1983; Sherry & Holmes 1988; Greenberg et al. 1999; Murakami 2002). Among them, I found only two studies considering the hypothesis of interspecific competition as being in the origin of such habitat divergence between species (Landres & MacMahon 1983; Sherry & Holmes 1988). Landres and MacMohon (1983) studied the North American bird communities in oak forests, while Sherry and Holmes (1988) conducted a more specific work with only two species of North American passerine from different families: Parulidae and Tyrannidae. Moreover, only Sherry and Holmes (1988) evaluated the coexistence of species at different spatial scales which highlights the singularity of this study. The existence of a habitat-shift between distantly related species may have a role in community structure which in general has been seldom taken in consideration, and thus should receive more attention from researchers on ecology and evolution.

In chapter 3, I measured the aggressive behaviour of two species differing in their territorial behaviour outside the breeding season: the territorial robin and the non-territorial blackcap. When territories are not defensible, mechanisms allowing for decisions over disputes with a minimum risk of injury are expected to evolve, such as the establishment of dominance ranks and status signalling (Lange & Leimar 2003; Pryke & Griffith 2006; Peiman & Robinson 2010). Our experiment showed that these species frequently engage in aggressive interactions. Robins and blackcaps presented

similar willingness to approach conspecific and heterospecific intruders, which is not in agreement with the prediction of uncertainly hypothesis (UcH), as uncertainty of injuries and fighting costs is expected to be higher among heterospecifics (Peiman & Robinson 2010). Moreover, the fact that there are large differences in plumage pattern between our species excludes the possibility of rival misidentification (Martin et al. 1996; Jones et al. 2016). Regarding the frequency of aggressive behaviours, conspecific aggressiveness was more intense than heterospecific aggressiveness in robins but not in blackcaps. These results suggested variable support for the UcH in the two species: aggressiveness in robins supported the hypothesis, since they displayed and attacked more frequently conspecifics than heterospecifics; on the contrary, aggressiveness in blackcaps did not support the hypothesis. The difference in agonistic behaviour between the two species may be related with the fact that robins are territorial in winter grounds whereas blackcaps forage opportunistically and do not incur costs of territorial defence (Pérez-Tris & Tellería 2002a). The large effort in territory defence may lead robins to defend food and space more frequently against individuals with greater overlap in resource preferences (i.e. conspecifics) than against heterospecifics, such as blackcaps. Our results (1) indicate the existence of interspecific competition between these two species, which is in agreement with the results from habitat use at a micro-scale (chapter 2), and (2) suggest it is worth investigating interspecific aggression even in phylogenetically-distant species, because it may be a relevant and selective pressure acting on their natural populations.

I also found that the eco-morphology of species can contribute to understanding the competition between them. In chapter 4, I measured the morphological overlap between an introduced species – the leiothrix – and the community of native species. Some studies found that exotic species obtain superior competitiveness over native species as a result of a greater foraging efficiency and exploratory ability (Petren & Case 1996; Piet 1998; Rehage & Sih 2004). The exotic leiothrix occupies the same morphological space which is occupied by the robin (and also, in a lesser extent, by the blackcap), indicating an establishment through competition rather than through opportunism (Sol et al. 2012; Batalha et al. 2013; Azzurro et al. 2014). Accordingly, the leiothrix has a wider gape and more rounded wings than the robin and the blackcap. These characteristics are indicative of foraging efficiency in woodlands (Morrison 1982; Keast

1996; Dawson 2005; Carnicer et al. 2009), which may contribute to a dominant competitiveness of the leiothrix over the native species considering their ecological similarities. Most research on the morphology of introduced birds has been focused in the coexistence of congeneric species (Moulton 1985), on the morphological space available in the receiving community (Lockwood et al. 1993; Batalha et al. 2013) or in the divergence of morphological characteristics after the introduction (Mathys & Lockwood 2011). Accordingly, these results can be considered quite novel since we found a possible advantageous effect of morphological traits of a colonizer species within a bird community.

Considering the results obtained in chapter 4, it was important to evaluate if a species with a more efficient eco-morphology was also a behaviourally dominant species. Therefore, in chapter 5, I measured the heterospecific dominance over a feeding context between the leiothrix and two native species: the robin and the blackcap. My experimental tests in feeding context revealed that the leiothrix was dominant over the robin and the blackcap. The leiothrix individuals were the winners in all tests against opponents of the two native species. However, the dominance of the leiothrix did not seem to result from greater aggressiveness of the exotic species, as no differences in aggressiveness were found in native-exotic interactions. Alternatively, other possible explanations for its dominance are a larger body size, greater weaponry or other non-agonistic behavioural traits that were not measured in our study (e.g. boldness, exploratory behaviour; Chase & Seitz 2011). The larger body size of the leiothrix suggested its dominance over the two smaller native species, as larger individuals tend to win aggressive interactions (Enquist & Leimar 1983). The weaponry size of the three study species does not appear to be independent on their body size and apparently has same relevance for the individual strength (as in other groups of birds; Martin & Ghalambor 2014). Accordingly, in most interactions, the aggressiveness by the native species was inconsequential as it did not lead to the displacement of the invasive species, and therefore it was insufficient to achieve dominance over the leiothrix. To the best of our knowledge, this is the first experimental work where clear dominance by an exotic bird species over native rivals was confirmed in a feeding context. Our work is also original considering the difference between levels of aggressiveness and dominance outcomes. Considering that subordinate species are likely to avoid dominant species and

reduce the signals which induce aggressiveness from dominant opponents (Martin et al. 2017), the relatively high levels of aggressiveness from native species that we found suggests that these particular native species were not capable of dealing with the presence of the invader. This can have negative consequences for the native species, such as an increased risk of injury.

Previously, I demonstrated the occurrence of habitat shifting between distantly related species (chapter 2), measured their heterospecific aggressiveness when they differ in the territorial effort (chapter 3), evaluated the existence of advantageous morphological characteristics in the interspecific competition (chapter 4), and measured the interspecific dominance of species in a feeding context (chapter 5). However, the intensity of aggressiveness can change according to social context (Muller & Wrangham 2004; Taillon & Côté 2007). Therefore, in chapter 6, I decided to measure the interspecific competition directly in the individual territories of the two native species during their breeding season by simulating territorial intrusions. I used their singing behaviour to measure the aggressive intensity of their responses. We submitted the robin and the blackcap (which are both territorial during the breeding season) to simulated territorial intrusions to assess the occurrence of interspecific aggression resulting from direct competition between two distantly related passerine species. From the results of other chapters, I already knew that the robin and the blackcap show habitat shifting during the breeding season (chapter 2) and that they use aggressive behaviour when submitted to a feeding experiment (chapter 3). However, it was important to study if both species also use their singing behaviour to show aggressiveness towards their opponents. In order to make the comparison more robust, we considered two other species that are either unlikely or new competitors: the non-competitor great tit (*Parus major*) and the exotic competitor red-billed leiothrix, which has a more efficient morphology than the robin and the blackcap and is a dominant species over them (chapters 4 and 5). The results of the chapter 6 indicated that the robin and the blackcap were differently affected by the species of the song playback, suggesting the use of different degrees of aggressiveness according to the species performing the territorial intrusion. The response of the robin was more intense towards the blackcap playback then the response measured in the reverse experiment. Since the motivational state of individuals can increase their aggressive behaviour (Gabor & Jaeger 1995; Arnott &

Elwood 2008), this asymmetric response between species may suggest differences in their habitat requirements. Accordingly, the robin has more limiting habitat requirements than the blackcap which can contribute to increase their aggressive response of robins towards blackcaps (as discussed in the chapter 2). The similar response of the focal birds to the leiothrix and the great tit (a non-competitor) can indicate the absence of rival-recognition of the leiothrix. This can be considered an expected result, since when species evolved in isolation (as the case of our native species and the exotic leiothrix), they are unable to initially recognize each other as rivals (Le Breton et al. 2007; Grether et al. 2009). However, some behavioural parameters measured in the focal species seems to indicate a subordinate response to the exotic competitor (i.e. showing greater latency to sing after the playback or spending fewer time closer to the speaker). This study demonstrated that some species of birds can use the song to signalize aggressiveness towards distantly related species. More specifically, the study also supported the hypothesis of interspecific competition between the robin and the blackcap which has been discussed in other chapters (see habitat shifting in chapter 2 and heterospecific aggressiveness in chapter 3). Moreover, it can be considered a quite innovative approach since we evaluated the heterospecific aggressiveness from the native-species perspective in opposition to other studies which used the exotic species as the aggressor (Lowe et al. 2011; Grundy et al. 2014; Hernández-Brito et al. 2014).

Conclusions

The main conclusion of this thesis is that interspecific competition can occur between distantly related species and that it is possible to measure it. Recent studies focused on interspecific competition, or more specifically on heterospecific aggressiveness, include large data sets from bird species from the same taxonomic family (e.g. Losin et al. 2016; Martin et al. 2017). However, species coexistence and the way they use the resources can be more important than the phylogenetic distance between species. I selected three bird species which rarely coexist with closely-relative species. However, I found some evidences of interspecific competition among them. By using the robin and the blackcap as models to study interspecific competition, I demonstrated that distantly

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related species that coevolved and coexist in the same habitats can show important levels of heterospecific aggression – sometimes as intense as among conspecifics – and developed some mechanisms to reduce interspecific competition, such as habitat shift. It would be interesting to use an optimality approach to obtain a more quantitative measure of the degree of interspecific competition between these two species, by manipulating resource defensibility and abundance. However, while the robin and the blackcap developed different fruit capture techniques to allow their coexistence while keeping similar fruit preferences (Jordano 1982; Rey et al. 1997; Herrera 1998), the exotic leiothrix is a newcomer with unpredictable foraging abilities. Here, I demonstrated that the leiothrix is dominant over native species, such as the robin and the blackcap, which perhaps contributes to justify why it is becoming established so rapidly in Europe. This can rise a conservation problem, considering that the dominance of an exotic species can affect negatively the population size of the subordinate native species (Gowaty 1984; Petren & Case 1996).

This work highlights (1) the important role of interspecific competition in the use of space between distantly related species that coevolved and (2) the ability of the native species to deal with a competing newcomer. In the future, it would be worth to measure some behavioural characteristics of these insectivorous passerines (such as aggressiveness, dominance and foraging behaviour) in conditions of allopatry and sympatry with their competitors. This work is a contribution to our understanding of interspecific competition in species evolution and how populations adjust to competition.
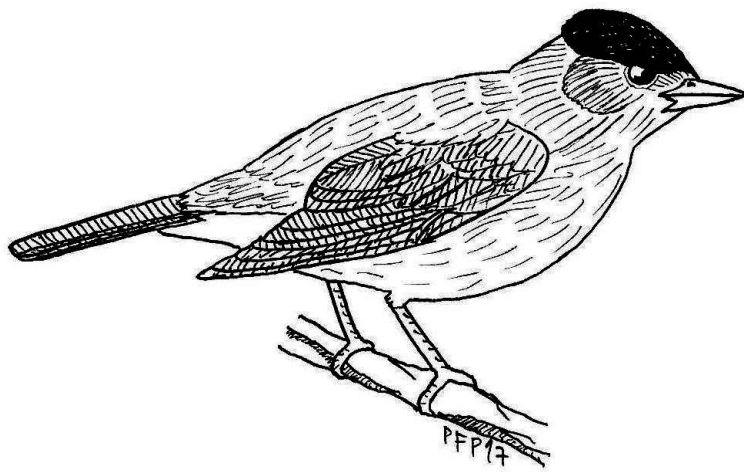
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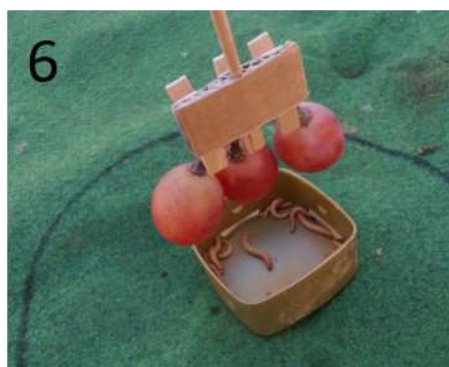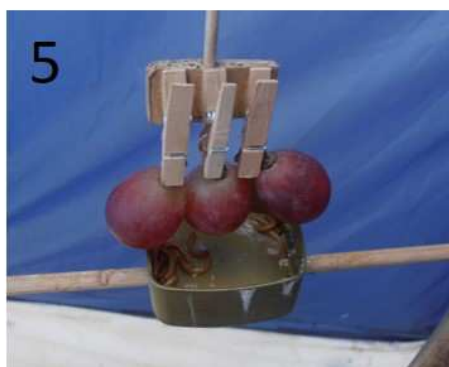
Appendix I

Fig. 1-3: Wood-types studied in chapter 2, (Fig. 1) exotic woods, (Fig. 2) pinewoods and (Fig. 3) native broadleaf woods; **Fig. 4:** Scheme of a transect used to measure the habitat of the robin and the blackcap at two scales: the landscape (the whole transect) and the site (an area within a circle of 10 m radius). The number of individuals (per each species) and their positions within the transect are illustrative.
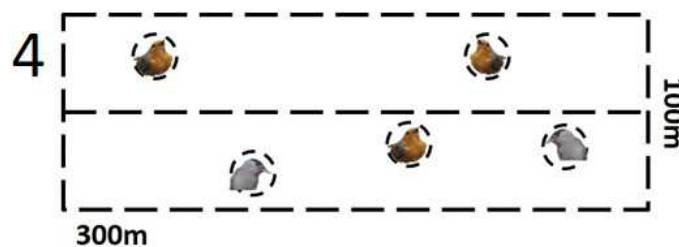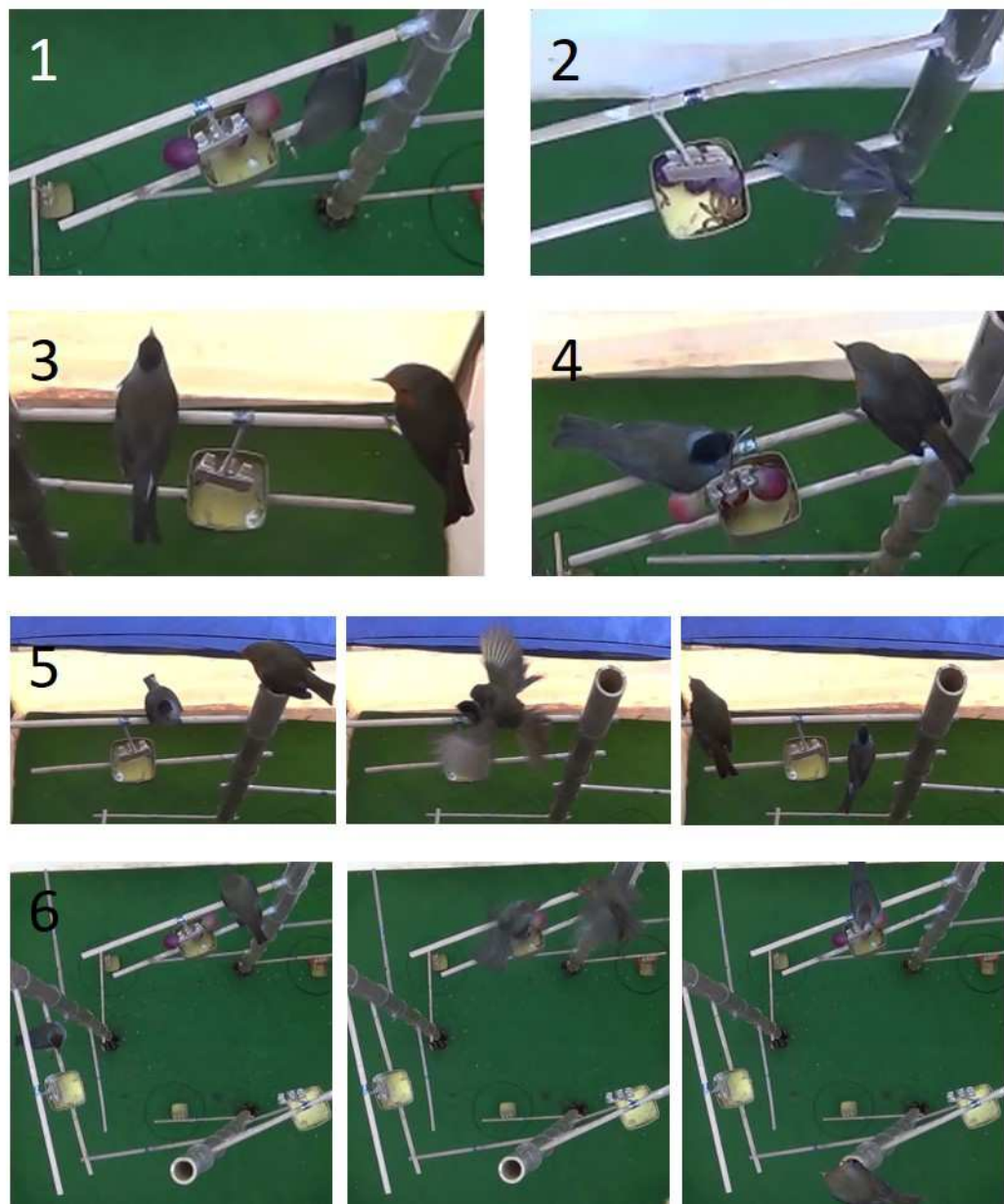
Appendix II

Fig. 1: Controlled environment (tent) where the experiments of chapters 3-5 were performed (base: 1.40 x 1.40 m, height 2.70 m); **Fig. 2:** Location of the video camera at the tent top; **Fig. 3-4:** Views of the three artificial wood trees (height 1.7 m) with five wood branches (0.30 m long); **Fig. 5-6:** Feeders containing grapes and mealworms located in a branch (Fig. 5) and on the ground (Fig. 6) used in the experiments of chapters 3 and 5.

Appendix III

Fig. 1-2: Birds feeding inside the experimental tent: a robin (Fig. 1) and a blackcap (Fig. 2); **Fig. 3:** A robin using a lower-intensity head forward display (D1 display studied in the chapter 3) towards a blackcap; **Fig. 4:** A blackcap using a higher-intensity head forward display (D2 display studied in the chapter 3) towards a robin; **Fig. 5-6:** Attacking behaviour between robins and blackcaps represented by two examples of three sequential frames (from the left to the right). A robin attacking a blackcap (Fig. 5) and the reverse (Fig. 6).

Appendix IV

Fig. 1: Habitat where the playback experiments were performed (chapter 6); **Fig. 2-5:** Dummies used in the simulated territorial intrusions of the playback experiments: robin (Fig. 2), blackcap (Fig. 3), great tit (Fig. 4) and red-billed leiothrix (Fig. 5); **Fig. 6:** General view of the apparatus used in the simulated territorial intrusions showing a dummy and the loudspeaker used to broadcast the song playbacks.