Breeding ecology and dispersal capability of wetland birds:

a comparison between two *Acrocephalus* warblers with unsynchronized life histories

Tesis doctoral

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Cover: juvenile moustached warbler *Acrocephalus melanopogon*, photo by F. Ceresa

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Abstract

The moustached warbler *Acrocephalus melanopogon* and the reed warbler *Acrocephalus scirpaceus* are two insectivorous reedbed nesting passerines. These species are very similar in size, but they differ in several ecological and behavioural aspects, such as migration strategy, breeding phenology and habitat specialization. In this work, we studied mainly Spanish populations of the two species, and our aims were to investigate some aspects of their breeding ecology, to assess their genetic population structure and dispersal ability, and to investigate their niche overlap at a sympatry breeding site. We were especially interested in assessing if the ecological and behavioural differences between the two species influence their dispersal ability and how they coexist when breeding in sympatry. We found that the two species are potential competitors for food, and this may explain the reduced spatial and temporal overlap we observed between the two species during the breeding season. This suggests that exploitative competition for food may play a more important role within assemblages of reedbed nesting passerines than previously hypothesized. The food samples used to assess the diet of birds were obtained by using apomorphine as an emetic on the study species, and we found no evidence of harmful effects on birds after treatment according to survival probability and mass change. Using microsatellites and mitochondrial DNA data, we found evidences of gene flow among breeding sites for both study species, suggesting that these two warblers are able to compensate for habitat fragmentation. The genetic differentiation among sampling sites was lower in the reed than in the moustached warbler, possibly because of higher dispersal ability, lower philopatry, larger population size, more continuous breeding range or higher capability in crossing natural barriers of the first species. We found evidence for postglacial population growth in both study species, but such increasing and the colonisation of new areas occurred in partly different ways between the two warblers. We studied the dispersal ability of both species also by using ringing data from the Spanish marking scheme; the distribution of dispersal distances did not differ between the moustached and the reed warbler, as well as among age classes. We found cases of long distance dispersal for both species, up to more than 100 km. Such cases were more frequent than expected if the probability of settling of moving birds were constant. The similarity of dispersal patterns between the two species may be due to their phylogenetic relatedness and to similar evolutionary pressures, and is not consistent with the hypothesis of higher dispersal ability in long distance migrants than in sedentary/short distance migrant bird species.

Resumen

El carricerín real *Acrocephalus melanopogon* y el carricero común *Acrocephalus scirpaceus* son paseriformes insectívoros típicos de carrizal. Estas especies son muy similares en tamaño, pero difieren en muchas características ecológicas y etológicas, como migración, fenología reproductiva y especialización de hábitat. En este estudio, hemos considerado sobre todo poblaciones españolas de las dos especies, y nuestros objetivos fueron por un lado investigar algunos aspectos de la ecología reproductiva, la estructura genética y la capacidad de dispersión de estas poblaciones, y por otro estudiar el solapamiento de nichos entre las dos especies en condiciones de simpatría. Nos interesaba especialmente comprobar si las diferencias a nivel ecológico y etológico entre las dos especies afectan su capacidad de dispersión y como coexisten cuando se reproducen en condiciones de simpatría. Hemos comprobado que las dos especies son potenciales competidoras por la comida, algo que podría explicar el reducido solapamiento observado a nivel temporal y espacial durante la estación reproductiva. Esto sugiere que la competencia por la comida podría tener un papel más importante en los ensamblajes de paseriformes de los carrizales, en comparación con hipótesis anteriores. Las muestras de comida utilizadas para estudiar la dieta de las aves han sido obtenidas suministrado a las aves un emético, la apomorfina, y no hemos encontrado evidencias de efectos negativos sobre las aves después del tratamiento, ni en la probabilidad de supervivencia ni en la variación de masa. Utilizando datos de microsatélites y de ADN mitocondrial, hemos encontrado evidencias de flujo genético entre sitios de cría de ambas especies, y esto sugiere que tienen la capacidad de enfrentarse a la fragmentación de su hábitat. La diferenciación genética entre sitios de muestreo resultó ser menor en el carricero común que en el carricerín real, posiblemente debido a una mayor capacidad de dispersión, a un rango de distribución más continuo en el área de estudio, a un mayor tamaño de población, a una menor filopatría o a una mayor capacidad de cruzar barreras geográficas de la primera especie. En ambas especies hemos encontrado evidencias de un incremento post-glacial del tamaño de población, aunque dicho incremento y la colonización de nuevas áreas ocurrieron de manera parcialmente diferente. Hemos estudiado la capacidad de dispersión de las dos especies también utilizando datos de anillamiento recolectados en España; no hemos encontrado diferencias en la distribución de las distancias de dispersión entre el carricerín real y el carricero común, y tampoco entre clases de edad. En ambas especies hemos encontrado casos de dispersión de larga distancia, hasta más de 100 km. Estos casos ocurrieron con mayor frecuencia de lo que esperaríamos si la probabilidad de establecimiento de los individuos en dispersión fuese constante. La similitud entre los patrones de dispersión de las dos especies se podría explicar por su estrecha relación filogenética, o por las similares presiones evolutivas, y no es consistente con la hipótesis de que tienen una mayor capacidad de dispersión los migradores de larga distancia frente a las especies sedentarias/ migratorias de corta distancia.

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Introduction

Acrocephalus and *Locustella* warblers are passerine birds (Aves: Passeriformes) which typically breed in wet habitats such as swamps, lake and river banks, and wet grasslands (Kennerley & Pearson 2010). These small birds (~10-30 g) are mainly insectivorous and most species have a cryptic plumage consisting in pale underparts and brownish upperparts. The nine recognized species of *Locustella* warblers breed exclusively in the Palearctic, while genus *Acrocephalus* (37 species) also includes species breeding in sub-Saharan Africa, S Asia, Australia and several Pacific Ocean islands (Kennerley & Pearson 2010).

In Europe, *Acrocephalus* warblers dominate the assemblages of reedbed-nesting passerines, which often include also one or more species of *Locustella* warblers. In many wetlands, these warblers constitute a set of ecologically and morphologically similar coexisting species. Consequently, they are expected to show some degree of niche differentiation (Begon et al. 2006), such as interspecific differences in spatial or temporal distribution or in the use of trophic resources. In fact, some authors found differences in habitat preferences among coexisting *Acrocephalus* species (e.g., Hoi et al. 1991, Poulin et al. 2002), and there are also more or less pronounced differences in their breeding phenologies (Kennerley & Pearson 2010), while the available information about trophic niche overlap is scarce. Studying the breeding ecology of sympatric *Acrocephalus* species, taking into account also their use of food resources and the temporal dynamic of prey availability, may provide a better understanding of their co-occurrence and insights about the role of interspecific competition for food (exploitative competition). This last topic has been scarcely investigated so far, possibly because reedbeds are highly productive habitats (Whittaker 1975), thus food availability seemed unlikely to be scarce compared to food requirements. Nonetheless, Poulin et al. (2002) found a significant positive effect of prey availability on the abundance of reedbed-nesting passerines, result that is not consistent with a general overabundance of preys. Until now, most studies about the interspecific interactions among *Acrocephalus* warblers were focused on interference competition (Murray 1971, Catchpole 1978, Hoi et al. 1991, Honza et al. 1999). While the large-sized great reed warbler *Acrocephalus arundinaceus* can influence the distribution of smaller *Acrocephalus* (also by destroying their nests; Hoi et al. 1991, Honza et al. 1999), such studies did not provide clear results for other species.

Besides the high productivity mentioned above, reedbeds are also characterized by high patchiness, which is both a natural feature and the result of wetlands destruction due to human activities (Van Vessem et al. 1997, Laurence 2010). In such a discontinuous habitat, the capability to disperse is a crucial characteristic to produce sufficient gene flow among spatially isolated breeding areas (Frankham et al. 2010). If a population become small and isolated, the probability of inbreeding and genetic drift increases, and these effects may imply an increase in extinction risk (Young & Clarke 2000, Frankham et al. 2010). Small and isolated populations are also more vulnerable to stochastic variations in demographic parameters (birth and death rates, sex ratio), catastrophic events like large fires or floods, and environmental fluctuations (e.g., climatic variability; Bennett & Saunders 2010). Birds have generally a high dispersal ability (Koenig et al. 1996), but with important interspecific differences which seem to be related, in a complex and still poorly understood way, to several species' characteristics (e.g., migration strategy, population size, distribution range, breeding habitat) and to their phylogenetic relatedness (Paradis et al. 1998, Sutherland et al. 2000). Additional data and comparative studies are needed to improve our understanding of dispersal in birds. However, in species with high active displacement ability, dispersal outside the natal/breeding site is often difficult to study because very large study areas should be adopted to avoid the underestimation of long-distance dispersal events (Paradis et al. 1998). Such events are relatively rare, but they are likely to have important effects on population dynamics and on the genetic population structure (Ibrahim et al. 1996, Frankham et al. 2010). For this reason, some recent studies about dispersal distances in birds were carried out using mark-recapture data obtained over large regions or entire countries (Paradis et al. 1998, 2002, Winkler et al. 2005, Van Houtan et al. 2007, 2010). Information about dispersal capability has been often obtained also from genetic data, by assessing the genetic differentiation among populations or by detecting possible immigrant individuals within a population (Piry et al. 2004, Frankham et al. 2010).

The species taken into account in this thesis are the moustached warbler *Acrocephalus melanopogon* and the reed warbler *Acrocephalus scirpaceus*, and we present the results of studies concerning their breeding ecology, dispersal ability and genetic population structure. We considered the moustached and the reed warbler because this allows to compare two phylogenetically closely related species with several ecological and behavioural differences (see the following section for details). We were especially interested in assessing if such differences influence their dispersal ability and how they coexist when breeding in sympatry. Two other species, the great reed warbler and the Savi's warbler *Locustella luscinioides*, were considered only in one methodological part of this thesis, to assess the effects of using an emetic substance on birds to obtain food samples (see chapter 1). All these species typically breed in reedbeds (Kennerley & Pearson 2010), therefore they can be found breeding in sympatry, as occurs in part of our study area (mainly Spain, also Morocco in one study, see chapter 3).

Study species

The moustached warbler breeds across a wide but discontinuous area ranging from NW Africa to Kazakhstan (Kennerley & Pearson 2010). Three subspecies have been recognized: *A. m. melanopogon* (NW Africa, South and Central-East Europe, Low Danube and S Ukraine, W Turkey), *A. m. albiventris* (Lower Don and E Black Sea) and *A. m. mimicus* (from SE Turkey to Kazakhstan; Kennerley & Pearson 2010). The European population is estimated in 150000-300000 breeding pairs and it is considered to be stable, mainly because of the stability of the strongholds in Austria and Romania (BirdLife International 2004). The species is included in the Annex I of the Birds Directive (2009/147/EC). In Spain, it breeds mainly along the Mediterranean coast and in the Balearic Islands, and more scarcely in some inland marshlands of Extremadura, Castilla-La Mancha and Aragón; the population has been estimated in approximately 1000 breeding pairs, which represents a decline of more than 50% compared to the '90s decade (Castany & López 2006). Such decline has been probably due to habitat degradation in several wetlands (Castany & López 2006).

This species has specialized habitat requirements, breeding in flooded reedbeds and preferably where common reed *Phragmites australis* is mixed with other wetland plants such as *Typha*, *Scirpus*, *Cladium* and *Juncus* (Castany 2003, Kennerley & Pearson 2010). Some of these plants are vulnerable to reedbeds management practices such as winter cutting and variations in the water level, thus these practices are likely to negatively affect the moustached warbler (Poulin et al. 2002), possibly also by reducing prey availability (Schmidt et al. 2005). The diet is constituted by several types of insects and their larvae (Coleoptera, Hymenoptera, Hemipera, Ephemeroptera, Odonata, Lepidoptera, Trichoptera, Diptera), spiders (Araneae), woodlice (Isopoda), water snails (Planorbidae and Lymnaeidae), and occasionally fruits (Dyrcz 2016). Small spiders are reported to represent a large part of the diet in a Mediterranean marshland (Poulin et al. 2002).

Unlike the other *Acrocephalus* warblers breeding in Europe, which migrate over long distances, S Europe moustached warbler populations are sedentary or short-distances migratory, and birds breeding in Central and E Europe winter in the Mediterranean area (Kennerley & Pearson 2010). This warbler starts breeding earlier than other congeneric species, beginning to lay eggs in late March/April, according to the breeding area (Castany 2003, Kennerley & Pearson 2010). No information about the genetic population structure of this species was available before the studies presented in this thesis, and knowledge about its dispersal patterns was scarce; in Hungary, Vadász et al. (2008) found higher breeding site fidelity in this species than in other *Acrocephalus* warblers. In the nominal subspecies, both adults and young birds have a complete moult in summer-early autumn, at or near the breeding/natal areas, and also a pre-breeding partial moult (Kennerley & Pearson 2010).

The reed warbler is a very widespread bird breeding in Europe, Asia and Africa, with three recognized subspecies: *A. s. scirpaceus* (N Africa, Europe), *A. s. fuscus* (from Central and E Turkey to W China), and *A. s. avicenniae* (Red Sea area; Kennerley & Pearson 2010); these subspecies correspond to three evolutionary lineages, according to mitochondrial DNA data (Arbabi et al. 2014). The European population of this warbler has been estimated in 2.7-5 millions of breeding pairs and is considered to be stable (BirdLife International 2004). In Spain, the breeding population has been estimated in 20000-100000 pairs (BirdLife International 2004), mainly concentrated in the Ebro, Tajo and Guadalquivir valleys, along the Mediterranean coast and in the Northern Inland Plateau (Gainzarain 2003).

The reed warbler breeds preferably in mature inundated reedbeds, and can settle also in small patches and narrow strips of reeds, zones where reeds are mixed with bushes and dry reedbed areas (Poulin et al. 2002, Kennerley & Pearson 2010). The diet of this warbler includes a variety of insects and their larvae (Coleoptera, Hymenoptera, Hemipera, Ephemeroptera, Odonata, Lepidoptera, Trichoptera, Diptera, Psocoptera), spiders, harvestman (Opiliones), pseudoscorpions (Chelonethi), small molluscs (snails and bivalves), and occasionally fruits, flowers and seeds (Dyrcz et al. 2016). In England, Davies & Green (1976) found the nestlings' diet to be constituted mainly by dipterans (65%), while Bibby & Thomas (1985) reported that spiders, dipterans and beetles jointly represented scarcely more than the half of the nestlings' diet. Bibby & Thomas (1985) also measured prey availability and found a broad similarity with prey consumption, suggesting a generalist foraging behaviour, although some abundant prey types (like dipterans) were relatively underexploited. At a breeding site in Poland, the abundance of the main prey types showed peaks in different periods, resulting in high food availability during all the breeding season (Dyrcz & Zdunek 1996).

Excepted some N African populations, birds of the nominal subspecies are long-distance migrants and winter in sub-Saharan Africa (Kennerley & Pearson 2010). A migratory divide in Central Europe separates the populations of the nominal subspecies: starting from late July, birds breeding in W and N Europe migrate through the Iberian Peninsula and Morocco to reach sub-Saharan W Africa, while Czech, Hungarian and SE European populations reach more eastern wintering areas by crossing the eastern Mediterranean (Procházka et al. 2008). Reed warblers arrive back to Europe between March and June, according to the breeding area; in SW Europe most of arrivals take place in April and egg-laying occurs mainly between May and July/August (Kennerley & Pearson 2010). In an area of Central Europe, between 1970 and 2006 the mean temperatures during the breeding season increased significantly, and both the beginning and the peak of egg laying occurred progressively earlier (Halupka et al. 2008). Furthermore, nesting success increased with temperatures, possibly because nests were better hidden by earlier developed reeds, or due to higher insect abundance (Halupka et al. 2008). The species shows a good natal and breeding dispersal capability (up to more than 250 km) according to mark-recapture data from the British population (Paradis et al. 1998). Genetic analyses on European populations showed low population differentiation, which confirms the high dispersal capability of reed warblers (Kralj et al. 2010, Procházka et al. 2011). In birds belonging to the nominate subspecies, both young and adult birds perform a partial moult in late summer and a complete moult in winter (Kennerley & Pearson 2010). Some Iberian birds have been found to moult completely in their breeding grounds, and this also occurs regularly in the Moroccan breeding population (Kennerley & Pearson 2010).

Concerning the niche overlap and the interactions between the moustached and the reed warbler, until now their diet overlap was never assessed, while some authors compared their spatial patterns: in a marshland of Central Europe Hoi et al. (1991) did not find evidence for dominant relationships between the two species. In the Mediterranean area, other researchers found possible signs of competition: Castany (2003) found a surprisingly low breeding density of reed warblers in a wetland hosting a large population of moustached warblers; both Poulin et al. (2002) and Castany (2003) observed that reed warblers were abundant in reedbed areas not suitable for other species. Thus, the reed warbler possibly take advantage of its high adaptability to settle in reedbed zones were potential competitors are scarce.

The Savi's and the great reed warbler, considered only in one chapter of this thesis as previously mentioned, are both long-distance migrants wintering in sub-Saharan Africa and are both insectivorous (Kennerley & Pearson 2010). These two warblers mainly breed in reedbeds and are distributed across large areas of Europe, and also in some zones of N Africa, but the Savi's warbler has more specialized habitat requirements, and it is less common than the great reed warbler both in Europe and in Spain (BirdLife International 2004).

Objectives

The aim of this thesis was to study the breeding ecology of moustached and reed warblers at a sympatry breeding site and to investigate their dispersal ability and genetic population structure, considering mainly Spanish populations; we then compared the results obtained for both species, taking into account their ecological and behavioural differences. More specifically, our objectives were:

1. To assess the diet, the prey selection, and the temporal trend of prey availability and prey need during the breeding season.
2. To contribute in finding an effective and safe field technique to obtain samples of regurgitated food from passerine birds.
3. To investigate, at a sympatry breeding site, the niche overlap of the two study species, and based on this to obtain insights about their interspecific interactions.
4. To verify the consequences of wetland fragmentation on the genetic diversity and population structure of the two study species.

5. To assess the natal and breeding dispersal capability of the two study species.
6. To compare the genetic population structure and the dispersal capability of the two species, taking into account their differences in migration strategy, population size and habitat specialization.

Brief description of the chapters

This thesis is organized as a series of papers, all of which have already been accepted for publication. The papers are not presented in chronological order, but follow a logical sequence based on the topics and the aims of each single work:

Chapter 1. Ceresa F., Belda E. J. & Monrós J. S. 2014. Apomorphine as an emetic for insectivorous songbirds: effectiveness and post-release effects on survival and mass change. Journal of Field Ornithology 85: 213-220.

Chapter 2. Ceresa F., Belda E. J., Gómez J., Miñana I. J., Soler L., Villarroya J. & Monrós J. S. 2016. Differences in time and space use between two sympatric *Acrocephalus* warblers with similar diets. Bird Study 63: 172-180.

Chapter 3. Ceresa F., Belda E. J., Kvist L., Rguibi-Idrissi H. & Monrós J. S. 2015. Does fragmentation of wetlands affect gene flow in sympatric *Acrocephalus* warblers with different migration strategies? Journal of Avian Biology 46: 577-588.

Chapter 4. Ceresa F., Belda E. J. & Monrós J. S. 2016. Similar dispersal patterns between two closely related birds with contrasting migration strategies. Population Ecology 58: 421-427.

Below, we provide a brief summary for each of the four chapters.

Chapter 1. To investigate the diet of our study species during the breeding season (see chapter 2) we needed food samples, thus we used apomorphine as an emetic on birds. In the literature this substance has been described as effective and apparently harmless, at least during and immediately after treatment (Valera et al. 1997, Poulin et al. 2002).

These characteristics were confirmed during our fieldwork, but we wanted to assess the occurrence of possible harmful effects of apomorphine on birds also after treatment. This kind of information about emetics is very scarce in the literature and may help researchers in choosing the best food sampling technique. Therefore, we compared the survival probability and mass change of treated and control birds during the weeks following treatment. This work represents the first assessment of the effects of emetics on birds using survival estimations based on the Cormak-Jolly-Seber model. In addition, we provided information about the effectiveness of the emetic; in this part, besides the moustached and the reed warbler, we also considered the Savi's and the great reed warbler.

Chapter 2. In this work we studied the breeding ecology of moustached and reed warbler at a sympatry site, the Pego-Oliva Natural Park (Mediterranean coast of Spain). We collected data about breeding phenology, diet, food availability and spatial distribution of the two species. Our aim was to assess their overlap in the use of time, space and food resources. We also described the prey selection and the temporal trends of food availability and food demand during the breeding season of both warblers. This study provided insights about the possibility of competitive interactions among the two study species.

Chapter 3. In this study we used both microsatellites (16 loci) and mitochondrial DNA to investigate the genetic population structure, genetic diversity and demographic history of moustached and reed warblers in areas where wetlands are highly fragmented. We were interested in comparing two closely related species, but with different migration strategies and different population sizes in the study area. Blood samples were collected at three breeding sites in Spain and, only for the reed warbler, one in Morocco. Before this study, no information about population structure and demographic history was available for the moustached warbler.

Chapter 4. After obtaining indirect information about dispersal ability of moustached and reed warblers (chapter 3), we looked for additional direct information using mark-recapture data from the Spanish ringing scheme. Our aims were to compare the distribution of dispersal distances between the two species and between age classes (natal vs breeding dispersal) and to find the best conceptual description for dispersal distances among a set alternative models (normal, exponential and Cauchy distributions). We then compared the results with the information obtained from genetic data (chapter 3) and discussed the role of dispersal capability, philopatry, population size, breeding range patchiness and geographical barriers in determining the observed patterns.

References

Arbabi T., Gonzalez J., Witt H.-H., Klein R. & Wink M. 2014. Mitochondrial phylogeography of the Eurasian Reed Warbler *Acrocephalus scirpaceus* and the first genetic record of *A. s. fuscus* in Central Europe. Ibis 156: 799-811.

Begon M., Townsend C. R. & Harper J. L. 2006. *Ecology: from individual to ecosystems*, 4th ed. Blackwell Publishing.

Bennett A. F. & Saunders D. A. 2010. Habitat fragmentation and landscape change. In: Sohdi N. S. & Ehrlich P. R., eds. *Conservation Biology for All*. Oxford University Press.

Bibby C. J. & Thomas D. K. 1985. Breeding and diets of the Reed Warbler at a rich and a poor site. Bird Study 32: 19-31.

BirdLife International. 2004. *Birds in Europe: population estimates, trends and conservation status*. BirdLife International.

Castany J. 2003. El carricerín real (*Acrocephalus melanopogon*) en el P. N. del Prat de Cabanes-Torreblanca. Doctoral thesis. University of Valencia.

Castany J. & López G. 2006. *El carricerín real en España. I Censo Nacional (2005)*. SEO/BirdLife.

Catchpole C. K. 1978. Interspecific territorialism and competition in *Acrocephalus* warblers as revealed by playback experiments in areas of sympatry and allopatry. Animal Behaviour 26: 1072-1080.

Davies N. B. & Green R. E. 1976. The development and ecological significance of feeding techniques in the reed warbler (*Acrocephalus scirpaceus*). Animal Behaviour 24: 213-229.

Dyrcz A. 2016. Moustached Warbler (*Acrocephalus melanopogon*). In: del Hoyo J., Elliott A., Sargatal J., Christie D.A. & de Juana E., eds. *Handbook of the Birds of the World Alive.* Lynx Edicions (retrieved from http://www.hbw.com/node/58792 on 29 July 2016).

Dyrcz A., Christie D. A. & Garcia E. F. J. 2016. Eurasian Reed warbler (*Acrocephalus scirpaceus*). In: del Hoyo J., Elliott A., Sargatal J., Christie D.A. & de Juana E., eds. *Handbook of the Birds of the World Alive.* Lynx Edicions (retrieved from http://www.hbw.com/node/58800 on 29 July 2016).

Dyrcz A. & Zdunek W. 1996. Potential food resources and nestling food in the great reed warbler *Acrocephalus arundinaceus* and reed warbler *Acrocephalus scirpaceus* at Milicz fishponds. Birds of Silesia 11: 123-132.

Frankham R., Ballou J. D. & Briscoe D. A. 2010. *Introduction to Conservation Genetics*, 2nd ed. Cambridge University Press.

Gainzarain J. A. 2003. Carricero común *Acrocephalus scirpaceus*. In: Martí R. & del Moral J. C., eds. *Atlas de las Aves Reproductoras de España*. DGCN-SEO/BirdLife.

Halupka L., Dyrcz A. & Borowiec M. 2008. Climate change affects breeding of reed warblers *Acrocephalus scirpaceus*. Journal of Avian Biology 39: 95-100.

Hoi H., Eichler T. & Dittami J. 1991. Territorial spacing and interspecific competition in three species of reed warblers. Oecologia 87: 443-448.

Honza M., Moksnes A., Roskraft E. & Øien I. J. 1999. Effect of great reed warbler Acrocephalus arundinaceus on the reproductive tactics of the reed warbler A. scirpaceus. Ibis 141: 489-493.

Ibrahim K. M., Nichols R. A. & Hewitt G. M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77: 282-291.

Kennerley P. & Pearson D. 2010. *Reed and Bush Warblers*. Christopher Helm Publishers Ltd.

Koenig W. D., Van Vuren D. & Hooge P. N. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends in Ecology & Evolution 11: 514 – 517.

Kralj J., Procházka P., Fainová D., Patzenhauerová H. & Tutiš V. 2010. Intraspecific variation in the wing shape and genetic differentiation of reed warblers *Acrocephalus scirpaceus* in Croatia. Acta Ornithologica 45: 51-58.

Laurence, W. F. 2010. Habitat destruction: death by a thousand cuts. In: Sohdi, N. S. & Ehrlich, P. R., eds. *Conservation biology for all*. Oxford University Press.

Murray B.G. 1971. The Ecological Consequences of Interspecific Territorial Bahavior in Birds. Ecology 52: 414-423.

Paradis E., Baillie S. R., Sutherland W.J. & Gregory R.D. 1998. Patterns of natal and breeding dispersal in birds. Journal of Animal Ecology 67: 518-536.

Paradis E., Baillie S. R. & Sutherland W. J. 2002. Modeling large-scale dispersal distances. Ecological Modelling 151: 279-292.

Piry S., Alapetite A., Cornuet J. M., Paetaku D., Baudouin L. & Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95: 536-539.

Poulin B., Lefebvre G. & Mauchamp A. 2002. Habitat requirements of passerines and reedbed management in southern France. Biological Conservation 107: 315-325.

Procházka P., Hobson K. A., Karcza Z. & Kralj J. 2008. Birds of a feather winter together: migratory connectivity in the reed warbler *Acrocephalus scirpaceus*. Journal of Ornithology 149: 141-150.

Procházka P., Stokke B. G., Jensen H., Fainová D., Bellinvia E., Fossøy F., Vikan J. R., Bryja J. & Soler M. 2011. Low genetic differentiation among reed warbler *Acrocephalus scirpaceus* populations across Europe. Journal of Avian Biology 42: 103-113.

Schmidt M. H., Lefebvre G., Poulin G. & Tscharntke T. 2005. reed cutting affects arthropods communities, potentially reducing food for passerine birds. Biological Conservation 121: 157-166.

Sutherland G. D., Harestad A. S., Price K., Lertzman K. P. 2000. Scaling of natal dispersal distances in terrestrial birds and mammals. Conservation Ecology 4:16 URL http://www.consecol.org/vol4/iss1/art16.

Vadász C., Német Á., Karcza Z., Loránt M., Biró C. & Csörgő T. 2008. Study on breeding site fidelity of *Acrocephalus* Warblers in Central Hungary. Acta Zoologica Academiae Scientiarum Hungaricae 54:167-175.

Valera F. J., Gutiérrez J. E. & Barrios R. 1997. Effectiveness, biases and mortality in the use of apomorphine for determining the diet of granivorous passerines. Condor 99: 765-772.

Van Houtan K. S., Pimm S. L., Halley J. M., Bierregaard R. O. Jr & Lovejoy T. E. 2007. Dispersal of Amazonian birds in continuous and fragmented forest. Ecology Letters 10: 219-229.

Van Houtan K. S., Bass O. L. Jr, Lockwood J. & Pimm S. L. 2010. Importance of estimating dispersal for endangered bird management. Conservation Letters 3: 260-266.

Van Vessem J., Hecker N. & Tucker G. M. 1997. Inland wetlands. In: Tucker, G. M. and Evans, M. I., eds. *Habitats for birds in Europe: A conservation strategy for the wider environment*. BirdLife Conservation Series 6. BirdLife International.

Whittaker R. H. 1975. *Communities and Ecosystems*. MacMillan.

Winkler D. W., Wrege P. H., Allen P. E., Kast T. L., Senesac P., Wasson M. F. & Sullivan P. J. 2005. The natal dispersal of tree swallows in a continuous mainland environment. Journal of Animal Ecology 74:1080-1090.

Young A. G. & Clarke M. 2000. *Genetics, Demography and Viability of Fragmented Populations*. Cambridge University Press.

Chapter 1

Apomorphine as an emetic for insectivorous songbirds: effectiveness and post-release effects on survival and mass change

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Apomorphine as an emetic for insectivorous songbirds: effectiveness and post-release effects on survival and mass change

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ABSTRACT. Emetics can be used to obtain food samples from birds, but they can harm birds during or after treatment. Studies to date suggest that apomorphine is a safe emetic for songbirds, but information is needed about possible post-release deleterious effects. From March to July 2012, we collected food samples from insectivorous songbirds using apomorphine. We treated 67 Moustached Warblers (*Acrocephalus melanopogon*), 56 Reed Warblers (*Acrocephalus scirpaceus*), 15 Great Reed Warblers (*Acrocephalus arundinaceus*), and 12 Savi's Warblers (*Locustella luscinoides*). Effectiveness in inducing regurgitation was high (76.7%) and varied among species, being significantly more effective with Reed Warblers (91.1%). No birds died during treatment. To check for possible post-release negative effects, we considered 53 treated Moustached Warblers and 37 treated Reed Warblers and selected an equal number of untreated individuals (simply captured, banded, and measured). We found no support for differences in survival or recapture probabilities between treated and untreated birds of either species within 21 d after administering apomorphine. We calculated body mass changes of all Moustached Warblers subsequently recaptured (within 21 d) and found no difference between treated ($N = 8$) and untreated ($N = 22$) birds, suggesting normal foraging activity after release. Our results suggest that apomorphine is a safe emetic, with no negative effect on survival at least in the short term. The effectiveness of apomorphine with insectivorous songbirds in our study contrasts with the results of some previous studies and confirms the differences in effectiveness among different taxa of songbirds. As with differences in effectiveness among species in our study, this variability in sensitivity to the emetic could be caused by morphological and physiological differences among different taxa.

RESUMEN. **Apomorfina como emético para aves canoras insectívoras: efectividad, efectos post-liberación, sobrevivencia y cambio en masa corporal**
Los eméticos pueden ser utilizados para obtener muestras de lo que ingieren las aves, pero pueden causarle daño a estas durante y después del tratamiento. Estudios han sugerido que la apomorfina puede ser un emético seguro para aves canoras, pero se necesita información sobre sus posibles efectos adversos una vez se liberan las aves. De marzo – julio 2012, obtuvimos muestras de lo ingerido por aves canoras insectívoras utilizando apomorfina. Usamos este como emético en 67 individuos de *Acrocephalus melanopogon*, 56 de *A. scirpaceus*, 15 de *A. arundinaceus* y 12 de *Locustella luscinoides*. La efectividad para inducir la regurgitación fue alta (76.6%) y varió entre especies, siendo significativamente más efectiva en *Locustella luscinoides* (91.1%). Ningún ave murió durante el procedimiento. Para examinar el posible efecto negativo post-liberación de las aves tomamos 53 individuos de *Acrocephalus melanopogon*, 37 individuos de *A. arundinaceus* y un número igual de individuos, que tan solo fueron capturados, anillados y medidos. No encontramos evidencia de diferencias en la sobrevivencia o probabilidad de recaptura entre aves tratados y no-tratadas, unos 21 días después de administrar la apomorfina. Calculamos el cambio en masa corporal de *Acrocephalus melanopogon*, (dentro de los 21 días subsiguientes a la captura) y no encontramos diferencias entre las aves tratadas ($N = 8$) y las no tratadas ($N = 22$), lo que sugiere actividades normales de forrajeo luego que se liberaron las aves. Nuestros resultados sugieren que la apomorfina es un emético seguro, sin efectos negativos en la sobrevivencia de las aves, a corto alcance. De nuestro estudio se desprende, que la efectividad de la apomorfina en aves canoras insectívoras contrasta con los resultados de otros trabajos y confirma diferencias en la efectividad entre diferentes taxones de aves canoras. Con respecto a las diferencias en el grado de efectividad (entre especies), encontrado en nuestro estudio, las diferencias en la sensibilidad al emético, pudieran estar asociados a diferencias morfológicas y fisiológicas entre diferentes taxones.

Key words: bird diet, capture-recapture analysis, Cormack-Jolly-Seber model, Moustached Warbler, Reed Warbler

Effectiveness in inducing regurgitation and the safety of different emetics can be influenced by many variables, such as dosage, bird size, bird species, stress during handling, and the amount of food in digestive tracts (Lederer and Crane 1978, Díaz 1989, Poulin et al. 1994, Poulin and Lefebvre 1995, Durães and Marini 2003,

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Diamond et al. 2007). Antimony potassium tartrate (tartar emetic) is a widely used emetic (Durães and Marini 2003). Despite being effective at inducing regurgitation (60.5–89.8% of treated birds; Poulin and Lefebvre 1995, Johnson et al. 2002, Durães and Marini 2003, Lopes et al. 2005, Carlisle and Holberton 2006, Diamond et al. 2007), several investigators have reported negative effects of this emetic on songbirds. For example, Zach and Falls (1976) reported mortality rates ranging from 12.5% to 50%, and Carlisle and Holberton (2006) reported that 1.5% of free-living and 94.4% (17 of 18) of captive Dark-eyed Juncos (*Junco hyemalis*) died after receiving the emetic. Poulin et al. (1994) treated a wide range of bird species and found an inverse relationship between mortality rate and body mass (higher mortality rates in birds <10 g), with significantly higher mortality rates for birds regurgitating only liquids (i.e., empty digestive tracts). However, other investigators have either reported no mortality (Tomback 1975, Zduniak 2005) of birds treated with tartar emetic or that mortality rates of small birds were no higher than those of larger birds (Poulin and Lefebvre 1995, Durães and Marini 2003). Other investigators have reported that reducing the concentration of the emetic reduced mortality rates of some especially sensitive small species (Poulin et al. 1994, Poulin and Lefebvre 1995).

Information about post-treatment deleterious effects of tartar emetic (e.g., death or abandonment of an area) in the wild is contradictory. For example, Johnson et al. (2002) reported significantly lower resighting rates of treated than untreated birds for three species of warblers (61.5% vs. 13.2%), whereas Poulin et al. (1994), Durães and Marini (2003), and Carlisle and Holberton (2006) found no significant differences between return rates of treated and untreated birds. Abandonment of an area due to the stress of treatment could result in lower resighting or return rates. For example, Poulin et al. (1994) suggested that stress associated with administration of the emetic led many birds to leave the area and hypothesized that mortality and desertion both contributed to the lower (although not significant) return rates of treated birds.

Other substances proposed for use as emetics include ipecac, lukewarm water, and apomorphine. Diamond et al. (2007) report no mortality using ipecac on songbirds and suggested that its dosages were less likely to reach a toxic level than tartar emetic. Investigators studying food habits of songbirds by flushing stomachs with lukewarm water have reported either no mortality (Ford et al. 1982) or low mortality rates (0.36%, Jenni et al. 1990), and no significant differences in return rates of treated and untreated birds (Ford et al. 1982, Jenni et al. 1990).

Investigators using apomorphine have reported effectiveness ranging from 43.7% to 71% (Schluter 1988, Díaz 1989, Valera et al. 1997) and either no mortality prior to release (Schluter 1988, Díaz 1989, Valera et al. 1997, Poulin et al. 2002, Mwangomo et al. 2007) or low rates of mortality (Valera et al. 1997). Such results suggest that apomorphine is a safe emetic, but information is needed about possible post-release deleterious effects. Furthermore, the effectiveness of this emetic on songbirds has been shown to be highly variable (Díaz 1989, Pulido and Díaz 1994, Valera et al. 1997, Poulin et al. 2002, Mwangomo et al. 2007) so species-specific investigations are warranted.

Our objective was to assess the impact of using apomorphine on several small (range = ~10–30 g) insectivorous songbirds, including Great Reed Warblers (*Acrocephalus arundinaceus*), Reed Warblers (*Acrocephalus scirpaceus*), Moustached Warblers (*Acrocephalus melanopogon*), and Savi's Warblers (*Locustella luscinoides*). In addition, for Moustached Warblers and Reed Warblers, we compared the survival, recapture probabilities, and mass of treated and untreated birds to provide further information about the effectiveness and safety of this emetic.

METHODS

Field work took place at the Pego-Oliva Natural Park (38°52′N 0°04′W, Spain) from 23 February 2012 to 5 July 2012. This coastal marshland (1250 ha) is located between the provinces of Valencia and Alicante, and includes large areas of reedbeds dominated by *Phragmites australis* and *Typha angustifolia*, and rice fields (Urios et al. 1993, Generalitat Valenciana 2010).

We established 10 capture stations in our study area, and captured birds daily using 6 mist-nets (10 m, 16-mm mesh) at one randomly selected station. Captures started 30 min before dawn and lasted 4 h. During normal banding activity, captured birds were banded, measured, aged, sexed when possible (Svensson 1992), weighed, and released.

During each of four periods (15–23 March, 10–30 April, 31 May–7 June, and 2–5 July), we collected food samples until we had treated at least 15 birds of each of the most common species (Moustached and Reed warblers). During each period, captured birds were banded, aged, and sexed, then two drops of a fresh saturated solution of apomorphine (0.04 g of hydrochloride hemihydrate per milliliter of water) were placed on each eye of a captured bird with a 1-ml pipette; birds were then held until the liquid was totally absorbed (~5 min; Valera et al. 1997, Poulin et al. 2002). Birds were then placed in a small, dark box lined with absorbent paper for 20 min (Valera et al. 1997), and we then measured and released them. We used apomorphine solutions for just 3 d to avoid potential loss of effectiveness (Díaz 1989) and checked for possible changes in effectiveness over time. We did not treat females that had brood patches to avoid possible harmful impacts, and other individuals were sometimes not treated either due to logistical problems (e.g., running out of apomorphine) or because we already had an adequate number of food samples. Untreated birds and those captured before and after food-sampling periods constituted the untreated set. These birds were simply banded, measured, aged, and sexed (when possible); they were not subjected to a real control procedure (i.e., putting two drops of distilled water on each eye and keeping them in the box for 20 min) due to logistical problems (e.g., too many birds to treat given the long procedure) and because we wanted to minimize possible negative impacts on captured birds. Therefore, treated birds were manipulated longer (~5 min to administer the emetic and 20 min in the box), with the consequent additional stress, than untreated birds. A chi-square (χ^2) test of independence was used to test for possible differences among species in the effectiveness of the emetic. Working on 2 × 2 contingency tables, chi-square values were subjected to Yates correction for continuity to avoid inflating Type I errors (Zar 2010). Apache OpenOffice Calc 3.4.1 (OpenOffice.org 2012) was used to perform the tests.

The possible effects on effectiveness of time since the apomorphine solution was prepared and time of capture were tested by fitting a saturated log-linear model to the three-way contingency table generated by the factors apomorphine effect (inducing regurgitation or not, 0/1), time since preparing the solution (first, second, and third day, corresponding to ~15, 39, and 63 h post-preparation, respectively), and time of capture (0–1.5, 1.5–3, and >3 h after opening mist-nets; Díaz et al. 1999). We could not include species as a factor in the model because that would have multiplied the number of cells by four, making the analysis unreliable. We used IBM SPSS Statistics 19.0 (Norušis 2011) to conduct the analysis.

To analyze possible differences in survival or recapture probabilities of treated and untreated birds, an untreated set was created for each species by selecting an equal number of untreated birds captured during the same period. We only considered Moustached and Reed warblers because sample sizes for the other two species were small ($N < 15$). We did not include the last sampling session (July; N treated birds = 14 Moustached Warblers and 19 Reed Warblers), given the lack of subsequent captures. We considered only recaptures from 1 to 21 d after capture to standardize capture effort. Over the 21-d period, captures were grouped into 7-d periods. This provided four capture periods: first capture, and recaptures during the first, second, and third weeks after the first capture, respectively. We analyzed capture-recapture data using models for open populations based on the Cormack-Jolly-Seber (CJS) model (e.g., Lebreton et al. 1992). These models produce survival estimates that are unbiased due to recapture probability. Hence, they are more reliable than those based only on return rates (Martin et al. 1995). Data were analyzed using MARK 5.1 software (White and Burnham 1999). The starting model was a model with time and group effects (treated vs. untreated) in both survival and recapture probabilities. The set of *a priori* models included all the possible models from the starting model to a model with constant survival and recapture probabilities (25 models). To determine if the data fulfilled assumptions of the CJS model, we used the bootstrap goodness-of-fit (GOF) test approach (1000 simulations). Bootstrap results were used to estimate overdispersion factor $\hat{c}$ (Burnham and Anderson 2002). Model selection was done using the corrected Akaike information criterion

Table 1. Number of birds treated with apomorphine and percent effectiveness of the emetic for each species.

Species	*N* treated	*N* regurgitated	% Effectiveness
Moustached Warbler	67	46	68.7
Reed Warbler	56	51	91.1
Great Reed Warbler	15	10	66.7
Savi's Warbler	12	8	66.7
Total	150	115	76.7

(AIC_c; see Burnham and Anderson 2002). We considered differences >2 AIC_c units to indicate a real difference in the fit of the model to the data (Burnham and Anderson 2002). AIC_c weights provide a relative measure of how well a model supports the data. We used model averaging to cope with model selection uncertainty. We used the Contrast program (Hines and Sauer 1989) to compare survival estimates.

To further assess the possible impact of apomorphine, we compared changes in mass of all treated and untreated Moustached Warblers that were subsequently recaptured and reweighed within 21 d. For analysis, we used a repeated-measures analysis of covariance (ANCOVA) with body mass as the repeated measure (mass at first capture and mass at recapture), treatment as the fixed factor, and tarsus length as the covariate to account for bird size (Freeman and Jackson 1990, Senar and Pascual 1997). We used IBM SPSS Statistics 19.0 (Norušis 2011) to conduct the analysis.

RESULTS

Of 150 birds that received the emetic, 115 (76.7%) regurgitated (Table 1). Four birds regurgitated only liquid, suggesting their stomachs were empty. To assess the usefulness of food samples, we performed a preliminary analysis by examining 19 samples from Moustached and Reed warblers. Mean mass of samples was 0.0029 ± 0.0031 (SD) g. We identified 6320 food fragments, with 2134 (34%) determined to be part of an organism (e.g., head, leg, antennae, thorax, or abdomen); unidentified fragments could not be identified as one of these parts. Using identified fragments, we identified all ingested arthropod prey and classified them to the order level.

No treated birds died, and all flew away when released. The emetic was more effective at inducing vomiting by Reed Warblers (51 of 56, 91.1%; Table 1) than by the other three species combined ($\chi^2_1 = 9.1$, $P = 0.0025$) and Moustached Warblers ($\chi^2_1 = 7.9$, $P = 0.0049$).

The results of fitting a saturated log linear model (Table 2) showed no significant interaction between effectiveness of the emetic and either time of capture or time since the apomorphine solution was prepared. All main effects (Effect, Time, and Day) were significant (Table 2), reflecting the high effectiveness of the emetic, the low number of birds treated during the second time interval compared to the first and third intervals, and the lower number of birds treated with a 3-d-old solution than with 1- or 2-d-old solutions.

Return rates of treated and untreated birds were 18.9% and 11.3% for Moustached Warblers and 10.8% and 8.1% for Reed Warblers, respectively (Table 3). For both species, the model that best fit the data was a model with constant survival and recapture probabilities (Table 4). The bootstrap GOF tests were not significant ($P = 0.47$ and $P = 0.91$, respectively). The constant model supports the hypothesis

Table 2. Results of the fit of a saturated log-linear model[a] including effect of apomorphine (Effect = not regurgitating = 0, regurgitating = 1), time of capture (Time = 0–1.5, 1.5–3, or >3 h after opening mist-nets), day since preparing the emetic (Day = first, second, or third day), and interactions between factors.

Parameter	Estimation	*Z*	*P*
Effect	−1.946	−2.2	0.026
Time	−1.946	−2.2	0.026
Day	0.963	2.7	0.008
Effect∗Day	0.136	0.1	0.89
Time∗Day	1.879	2.0	0.047
Effect∗Time	1.946	1.3	0.18
Effect∗Time∗Day	−1.291	−0.8	0.44

[a] Log-linear model: Constant + Effect + Time + Day + Effect∗Day + Time∗Day + Effect∗Time + Effect∗Time∗Day.

Table 3. The number of recaptured Moustached and Reed warblers in treated[a] and untreated groups.

	Captures			Recaptures within 21 d		
Species	*N* treated	*N* untreated	*N* total	*N* treated	*N* untreated	*N* total
Moustached Warbler	53	53	106	10 (18.9%)	6 (11.3%)	16 (15.1%)
Reed Warbler	37	37	74	4 (10.8%)	3 (8.1%)	7 (9.5%)
Total	90	90	180	14 (15.6%)	9 (10.0%)	23 (12.8%)

[a] Birds treated during the last sampling session (July; *N* treated birds = 14 Moustached Warblers and 19 Reed Warblers) are not included in the treated group, given the lack of subsequent captures.

Table 4. The top six CJS models estimating survival (ϕ) and recapture probability (*p*) of (a) Moustached Warblers and (b) Reed Warblers in relation to treatment group (*g*; treated/untreated) and time of capture (*t*), or with no group or time effect, i.e., constant (.) survival or recapture probability. For each model, values for corrected Akaike information criterion (AICc), the difference between that model and the model with the lowest AICc (ΔAICc), AICc weight, model likelihood, and number of estimable parameters are provided.

(a)

Model	AICc	ΔAICc	AICc weight	Model likelihood	*N* parameters
φ (.) *p* (.)	111.911	0.000	0.390	1.000	2
φ (g) *p* (.)	113.537	1.627	0.173	0.443	3
φ (.) *p* (g)	113.569	1.658	0.170	0.436	3
φ (.) *p* (t)	115.542	3.631	0.063	0.163	4
φ (g) *p* (g)	115.671	3.760	0.059	0.153	4
φ (t) *p* (.)	116.088	4.177	0.048	0.124	4

(b)

Model	AICc	ΔAICc	AICc weight	Model likelihood	*N* parameters
φ (.) *p* (.)	45.606	0.000	0.456	1.000	2
φ (.) *p* (g)	47.669	2.063	0.163	0.356	3
φ (g) *p* (.)	47.709	2.104	0.159	0.349	3
φ (.) *p* (t)	49.799	4.193	0.056	0.123	4
φ (g) *p* (g)	49.890	4.284	0.054	0.117	4
φ (t) *p* (.)	49.938	4.332	0.052	0.115	4

that there were no differences in the survival or recapture probabilities of treated and untreated groups. However, for Moustached Warblers, the second-best model included group effects in survival, but not in recapture, and there was a difference in AIC$_c$ with the constant model of 1.6 units (Table 4). Thus, given the model selection uncertainty, we used model averaging to estimate survival probabilities. Apparent weekly survival was 0.96 ± 0.23 (SE) for the treated group and 0.92 ± 0.25 for the untreated group, and this difference was not significant ($\chi^2_1 = 0.02$, $P = 0.90$; null hypothesis = homogeneous survival rates). The recapture probability for both treated and untreated groups using model averaging was 0.06 ± 0.03.

For Reed Warblers, the second-best model included differences in recapture probabilities between treated and untreated birds, and the difference in AIC$_c$ was >2 units (ΔAIC$_c$ = 2.1). For both treated and untreated groups, weekly survival probability estimated by model averaging was $0.99 \pm 0.08 \times 10^{-5}$ and recapture probability was 0.03 ± 0.01.

We obtained data on body mass changes for birds recaptured within 21 d for 8 treated (of 67 treated individuals) and 22 untreated birds (of 181 untreated individuals; Moustached Warblers only). The mean difference in body mass between first capture and recapture was 0.1 ± 0.4 (SD) g for treated birds and 0 ± 0.8 g for untreated birds, and this difference was not significant (repeated measures ANCOVA, $F_{1,27} = 0.1$, $P = 0.74$).

DISCUSSION

We obtained samples of stomach contents from 76.7% of birds treated with apomorphine. For all four species combined, the percentage of regurgitating birds was higher than that reported in studies of granivorous birds (range = 43.7–71%; see citations in the Introduction). However, apomorphine has been found to be ineffective with other insectivorous songbirds (Blue Tit, *Cyanistes caeruleus*, and Great Tit, *Parus major*; Pulido and Díaz 1994, Valera et al. 1997). Díaz (1989) also reported differences in the effectiveness of apomorphine among different families of granivorous passerines, and suggested that effectiveness was influenced by anatomical and physiological differences among taxa. These differences may include the mechanisms of emesis (e.g., sensitivity of the chemoreceptor trigger zone, see Chaney and Kare 1966), structure of the digestive tract (gastric and esophageal muscles), and the relationship between food-item size and bird size (e.g., seed fragments may be easier to regurgitate than intact ones; Díaz 1989). Nevertheless, our results and those reported by other investigators suggest that the effectiveness of apomorphine is similar to that of tartar emetic (range = 60.5–89.8%; see citations in the Introduction) and ipecac (68%; Diamond et al. 2007).

No treated birds died before release in our study, and other investigators using apomorphine have reported similar results (Schluter 1988, Díaz 1989, Valera et al. 1997, Poulin et al. 2002, Mwangomo et al. 2007). Investigators using lukewarm water (Ford et al. 1982) and ipecac (Diamond et al. 2007) as emetics have also reported no mortality, and others using lukewarm water reported extremely low mortality rates (Brensing 1977, Jenni et al. 1990). In contrast, for free-living birds treated with tartar emetic, reported mortality rates prior to release has ranged from 0% to 20% (Tomback 1975, Lederer and Crane 1978, Poulin et al. 1994, 2002, Poulin and Lefebvre 1995, Johnson et al. 2002, Durães and Marini 2003, Lopes et al. 2005, Zduniak 2005, Carlisle and Holberton 2006, Diamond et al. 2007). Diamond et al. (2007) suggested that use of non-optimal dosages and particularly stressful procedures could have contributed to an increase in the number of deaths in some studies.

We found no evidence of deleterious post-treatment effects, at least within a few weeks after treatment. Survival and recapture probabilities of treated and untreated birds were similar, as were changes in body mass of treated and untreated Moustached Warblers. Nevertheless, given our small sample sizes (especially the small number of recaptured birds) and lack of a real control procedure, additional studies with larger sample sizes are needed before concluding that apomorphine has no post-release effects on treated birds. Furthermore, because a real control procedure was lacking, we did not specifically test the toxic effect of the emetic, but the joint effect of apomorphine and the manipulation required to administer it (see Methods). Hence, we found no evidence of deleterious effects of the entire treatment. Using lukewarm water as an emetic, Ford et al. (1982) and Jenni et al. (1990) reported similar return rates for treated and untreated birds and, using ipecac, Diamond et al. (2007) reported a significantly higher return rate for treated than untreated birds (34% vs. 22%). Diamond et al. (2007) suggested, however, that this difference could have been due to differences in the species composition of treated and untreated groups. Investigators using tartar emetic have either reported significantly lower resighting rates for treated than untreated birds (Johnson et al. 2002) or no significant difference in return rates (Poulin et al. 1994, Durães and Marini 2003, Carlisle and Holberton 2006). However, Durães and Marini (2003) did not follow an experimental approach (i.e., untreated individuals were not randomly chosen), and Poulin et al. (1994) conducted their study on paired plots in which birds either did or did not receive the treatment, so that, as noted by Johnson et al. (2002), the effects of the emetic and study plot on return rates were confounded.

Considering pre-release mortality of songbirds, our results and those of previous studies indicate that apomorphine causes less mortality than tartar emetic. Available information about post-release deleterious effects of tartar emetic on free-living birds is contradictory (maybe because of the many variables influencing the effects of emetics), but studies of captive birds (Zach and Falls 1976, Carlisle and Holberton 2006) suggest that post-treatment effects can be serious, whereas our results and those of other investigators who used apomorphine with captive birds (Valera et al. 1997) suggest no negative post-treatment effects. Lukewarm water and ipecac have impacts similar to apomorphine both before and after treatment, and available information (especially about post-treatment effects) is not sufficient to determine which of these three substances should be considered safest for use with songbirds.

We conclude that apomorphine should be a preferred alternative to tartar emetic. However, effects of different emetics on birds can be influenced by many variables, such as dosage, bird size, bird species, stress during handling, and the amount of food in digestive tracts (see citations in the Introduction). Researchers using emetics should also take into account possible biases in representation of different food items in samples (Zach and Falls 1976, Gavett and Wakeley 1986, Valera et al. 1997). Additional studies with other species and larger samples are needed to better evaluate the possible post-treatment consequences of using apomorphine, especially a rigorous capture-recapture analysis that will allow estimates of survival and recapture probabilities.

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LITERATURE CITED

BRENSING, D. 1977. Nahrungsökologische Untersuchungen an Zugvögeln in einem südwestdeutschen Durchzugsgebiet während des Wegzuges. Vogelwarte 29: 44–56.

BURNHAM, K. P., AND D. R. ANDERSON. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Springer Verlag, New York, NY.

CARLISLE, J. D., AND R. L. HOLBERTON. 2006. Relative efficiency of fecal versus regurgitated samples for assessing diet and the deleterious effects of a tartar emetic on migratory birds. Journal of Field Ornithology 77: 126–135.

CHANEY, S. G., AND M. R. KARE. 1966. Emesis in birds. Journal of the American Veterinary Medical Association 149: 938–943.

DIAMOND, A. W., V. C. FAYAD, AND P. S. MCKINLEY. 2007. Ipecac: an improved emetic for wild birds. Journal of Field Ornithology 78: 436–439.

DÍAZ, M. 1989. Eficacia de un emético (apomorfina) para el estudio de las dietas de paseriformes granívoros. Ardeola 36: 185–191.

———, T. SANTOS, AND J. L. TELLERÍA. 1999. Effects of forest fragmentation on the winter body condition and population parameters of an habitat generalist, the wood mouse *Apodemus sylvaticus*: a test of hypotheses. Acta Oecologica 20: 39–49.

DURÃES, R., AND M. Â. MARINI. 2003. An evaluation of the use of tartar emetic in the study of bird diets in the Atlantic Forest of southeastern Brazil. Journal of Field Ornithology 74: 270–280.

FORD, H. A., N. FORDE, AND S. HARRINGTON. 1982. Non-destructive methods to determine the diets of birds. Corella 6: 6–10.

FREEMAN, S., AND W. M. JACKSON. 1990. Univariate metrics are not adequate to measure avian body size. Auk 107: 69–74.

GAVETT, A. P., AND J. S. WAKELEY. 1986. Diet of House Sparrows in urban and rural habitats. Wilson Bulletin 98: 137–144.

GENERALITAT VALENCIANA, CONSELLERIA DE INFRAESTRUCTURES, TERRITORI I MEDI AMBIENT [online]. 2010. <http://parquesnaturales.gva.es/web/indice.aspx?nodo=2988> (Accessed 14 November 2012).

HINES, J. E., AND J. R. SAUER. 1989. Program CONTRAST: a general program for the analysis of several survival or recovery rates estimates. Fish and Wildlife Technical Report 24, U.S. Fish and Wildlife Service, Washington, D.C.

JENNI, L., P. REUTIMANN, AND S. JENNI-EIERMANN. 1990. Recognizability of different food types in faeces and in alimentary flushes of *Sylvia* warblers. Ibis 132: 445–453.

JOHNSON, M. D., D. R. RUTHRAUFF, J. G. JONES, J. R. TIETZ, AND J. K. ROBINSON. 2002. Short-term effects of tartar emetic on re-sighting rates of migratory songbirds in the non-breeding season. Journal of Field Ornithology 73: 191–196.

LEBRETON, J.-D., K. P. BURNHAM, J. CLOBERT, AND D. R. ANDERSON. 1992. Modelling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecological Monographs 62: 67–118.

LEDERER, R. J., AND R. CRANE. 1978. The effects of emetics on wild birds. North American Bird Bander 3: 3–5.

LOPES, L. E., A. M. FERNANDES, AND M. Â. MARINI. 2005. Diet of some Atlantic Forest birds. Ararajuba 13: 95–103.

MARTIN, T. E., J. CLOBERT, AND D. R. ANDERSON. 1995. Return rates in studies of life history evolution: are biases large? Journal of Applied Statistics 22: 863–875.

MWANGOMO, E. A., L. H. HARDESTY, A. R. E. SINCLAIR, S. A. R. MDUMA, AND K. L. METZGER. 2007. Habitat selection, diet and interspecific associations of the Rufous-tailed Weaver and Fischer's Lovebird. African Journal of Ecology 46: 267–275.

NORUŠIS, M. J. 2011. IBM SPSS Statistics 19 guide to data analysis. Pearson Prentice-Hall, Upper Saddle River, NJ.

OPENOFFICE [online]. 2012. <http://www.openoffice.org/> (Accessed 13 September 2012)

POULIN, B., AND G. LEFEBVRE. 1995. Additional information on the use of tartar emetic in determining the diet of tropical birds. Condor 97: 897–902.

———, ———, AND A. MAUCHAMP. 2002. Habitat requirements of passerines and reedbed management in southern France. Biological Conservation 107: 315–325.

———, ———, AND R. MCNEIL. 1994. Effect and efficiency of tartar emetic in determining the diet of tropical land birds. Condor 96: 98–104.

PULIDO, F., AND M. DÍAZ. 1994. Diet and prey type selection by adult and young Blue Tits *Parus caeruleus*: the effect of correcting for prey digestibility. Ardeola 41: 151–160.

SCHLUTER, D. 1988. The evolution of finch communities on islands and continents: Kenya vs. Galápagos. Ecological Monographs 58: 229–249.

SENAR, J. C., AND J. PASCUAL. 1997. Keel and tarsus length may provide a good predictor of avian body size. Ardea 85: 269–274.

SVENSSON, L. 1992. Identification guide to European passerines, 4th ed. Svensson, Stockholm, Sweden.

TOMBACK, D. F. 1975. An emetic technique to investigate food preferences. Auk 92: 581–583.

URIOS, V., P. DONAT, AND M. J. VIÑALS. 1993. La marjal de Pego-Oliva. Instituto de estudios Comarcales de la Marina Alta, Valencia, Spain.

VALERA, F., J. E. GUTIÈRREZ, AND R. BARRIOS. 1997. Effectiveness, biases and mortality in the use of apomorphine for determining the diet of granivorous passerines. Condor 99: 765–772.

WHITE, G. C., AND K. P. BURNHAM. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46: 120–139.

ZACH, R., AND J. B. FALLS. 1976. Bias and mortality in the use of tartar emetic to determine the diet of Ovenbirds (Aves: Parulidae). Canadian Journal of Zoology 54: 1599–1603.

ZAR, J. H. 2010. Biostatistical analysis, 5th ed. Pearson Prentice-Hall, Upper Saddle River, NJ.

ZDUNIAK, P. 2005. Forced regurgitation with tartar emetic as an effective and safe method to study diet composition in Hooded Crow nestlings. European Journal of Wildlife Research 51: 122–125.

Differences in time and space use between two sympatric *Acrocephalus* warblers with similar diets

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Differences in time and space use between two sympatric *Acrocephalus* warblers with similar diets

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ABSTRACT

Capsule: We found high diet overlap and different uses of space and time between Moustached Warblers *Acrocephalus melanopogon* and Reed Warblers *Acrocephalus scirpaceus* breeding in sympatry at a marshland in Spain.

Aims: To study the degree of diet overlap between both species, their space use on a local scale and their breeding phenologies.

Methods: We studied the breeding phenologies of the two species by standardized ringing activity. Spatial distribution was investigated by point counts. We determined diet composition from emetic samples and we collected invertebrates by standardized sweep-netting to estimate food availability.

Results: Diet and prey selection were similar among species. Conversely, spatial overlap was relatively small (<50%) and breeding phenologies were not synchronized. Both food availability and the overall abundance of the two species increased throughout the breeding season.

Conclusion: The two species are potential competitors for food and the observed differences in spatial and temporal niches may represent a way to lower competition for trophic resources: Moustached Warblers could reduce competition by breeding early, while Reed Warblers could avoid settling in areas occupied by the other species.

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Studying the degree of niche overlap among morphologically and ecologically similar species is crucial to understand their co-occurrence (Gonzalez-Solis *et al.* 1997, Vieira & Port 2007). In fact, the coexistence of similar species is often associated with niche differentiation strategies, which include differences in resource use and in spatial and temporal distribution (Begon *et al.* 2006). Determining the degree of overlap in resource use provides information about the possibility of interspecific competition, although large overlap does not automatically imply competition (Wiens 1977). In fact a resource may be abundant enough, or even overabundant, compared to the overall demand of co-existing species. More generally, the occurrence of competition is difficult to assess when using merely observational data (Begon *et al.* 2006), and distribution models can highlight problems in understanding the factors influencing co-occurrence of species (Morelli & Tryjanowski 2015).

In this work, we studied two *Acrocephalus* warblers in a Spanish marshland where they breed sympatrically: the Moustached Warbler *Acrocephalus melanopogon* and the Reed Warbler *Acrocephalus scirpaceus*. Sympatric breeding populations of *Acrocephalus* species have been the subject of several studies of their interactions (Catchpole 1978, Hoi *et al.* 1991, Honza *et al.* 1999). Despite this, as far as we know there are currently no studies that have combined the temporal dynamics of reproduction and food availability. Moustached Warblers and Reed Warblers are extremely similar in size (~10 g) and both are insectivorous (Kennerley & Pearson 2010), but their migration strategy, reproduction timing and habitat specialization differ. South-west Mediterranean Moustached Warbler populations are sedentary or migrate over short distances, and start breeding earlier than the congeneric long-distance migrant species, with some females incubating as early as March (see our results and those of Castany 2003). This species has specialized breeding habitat preferences that require flooded reedbeds near open waters, and they prefer zones where *Phragmites* is mixed with other wetland plants like *Typha*, *Scirpus*, *Juncus* and *Cladium* (Castany 2003, Kennerley & Pearson 2010). The Reed Warbler is a long-distance migrant and winters in Africa,

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arriving back to Europe between March and May; in West Europe egg-laying begins in May (Kennerley & Pearson 2010). This warbler is a common and widespread breeding bird in European reedbeds, including those of limited extent (Kennerley & Pearson 2010). Flooded reedbeds are preferred, but breeding is also possible in drier habitats (Kennerley & Pearson 2010). In Spain, the Moustached Warbler breeds across a highly fragmented range, almost always in sympatry with the commoner Reed Warbler (Castany & Lopez 2006). As far as we know, the literature contains no detailed information about trophic niche overlap between the two species.

The aims of this work were to measure the trophic niche overlap between both species throughout the breeding season and to compare their spatial distributions and breeding phenologies in the study area. Given that potentially competing sympatric species are predicted to develop niche differentiation (Schoener 1974), we expected to find substantial differences in one or more of the investigated niche axes (trophic, spatial and temporal). To gain further insights into the coexistence of the two species, we used breeding phenology and arthropod abundance data to compare the temporal dynamics of food demand and food availability. We considered this species pair instead of a wider assemblage of sympatric reedbed-nesting insectivorous passerines because the remaining species (Great Reed Warbler *Acrocephalus arundinaceus* and Savi's Warbler *Locustella luscinioides*) were scarcer at our study site, thus we could obtain only low sample sizes.

Methods

Data collection

Data collection took place at the Pego-Oliva Natural Park (38°52′N 0°04′W), located on the Mediterranean coast of Spain. This marshland includes wide reedbed areas (dominated by *Phragmites australis* and *Typha angustifolia*), water bodies and rice fields, and covers approximately 1250 ha (Urios *et al.* 1993). Except for the point counts carried out in April 2013 and April 2014, all the fieldwork took place between 23 February and 5 July 2012.

We investigated breeding phenology by standardized ringing activity in a part of the marshland (30 ha) where both species breed. In this area we established ten ringing stations and captured birds daily with six mist nets (10 m, 16-mm mesh) at one randomly selected station. Captures started 30 minutes before dawn and lasted 4 hours. Birds were ringed, aged, sexed whenever possible (Svensson 1992), measured, weighed and released. The development of brood patches in breeding females was also recorded using the code proposed in Clarabuch (2000), and this information was used to estimate the higher food demand periods. Females with an evidently vascularized brood patch (codes 2 and 3, evident and maximum vascularization, respectively) were considered to be incubating eggs or rearing nestlings of up to 4–5 days old (Bailey 1952). According to Kennerley & Pearson (2010), incubation and the period from hatching to fledging respectively last 14–15 and approximately 12 days in Moustached Warblers, and 9–12 and 10–12 days in Reed Warblers. Accordingly, evident brood patch vascularization lasts approximately 18–20 days for Moustached Warblers and 13–17 days for Reed Warblers (incubation + 4–5 days after hatching). Thus we estimated the weekly abundance of pairs rearing nestlings as the average of abundances of the females with vascularized brood patches that were recorded in the previous 3 weeks. We considered the whole period of nestling rearing as the maximum food demand time. In fact, birds often have the greatest breeding success if they produce nestlings when food availability is seasonally at its highest (Lack 1968, Perrins & McCleery 1989, van Noordwijk *et al.* 1995). Ringing data were also used to measure the weekly overall abundance (Moustached + Reed Warblers) of the two species; that is, the abundance of potential competing individuals that forage at the same time in the reedbed. The resulting trend widely fluctuated, so we calculated the centred moving averages (MA) over 3 weeks to obtain more reliable information. In both cases (incubating females and Moustached + Reed Warblers) weekly abundance of birds was calculated as *N* captured birds/*N* days (usually 7, sometimes less due to adverse weather conditions which did not allow ringing activity).

We collected information about food availability by sampling invertebrates once a week through sweep-netting (Ausden & Drake 2006). Sampling took place 4 hours after dawn along four transects, located in the same area where we performed the ringing activity and always in the same places. These transects consisted of hitting vegetation with the net ring from the bottom upwards and alternatively on both sides of the trail (Poulin *et al.* 2002). Along each transect (approximately 125 m long), vegetation was swept 125 times, with 500 hits in all. We adopted this method because it samples a wide variety of invertebrate taxa from the foraging substrate of our study species (Poulin & Lefebvre 1997, Poulin *et al.* 2002). Afterwards, collected invertebrates were identified to the level of order according to Barrientos (2004) and were counted. Eggs, pupae and larvae were also counted, but were not taxonomically differentiated. A reference collection was created to facilitate prey identification (see below).

To study diet composition, during four periods (approximately monthly: 12–23 March, 10–30 April, 31 May–7 June and 2–5 July) birds captured when ringing were induced to regurgitate using apomorphine as an emetic agent (see Ceresa *et al.* 2014 for details). Each food sample was obtained from a different individual. Sampling periods lasted until we treated at least 15 individuals per species; from Moustached Warblers we did not always obtain this number of food samples because for this species the emetic was not highly effective (<70% of the treated individuals regurgitated, Ceresa *et al.* 2014). Overall, we obtained 44 food samples from Moustached Warblers and 48 from Reed Warblers. During the first sampling period, Reed Warblers were still absent in the study area. Thus we were able to sample only Moustached Warblers. According to the capture–recapture analysis and body mass change, we found no evidence for deleterious effects of this treatment on birds (Ceresa *et al.* 2014). We examined food samples under a stereo microscope and identified prey to the level of order according to Barrientos (2004) and Shiel *et al.* (1997), and also the reference collection created with the invertebrates sampled in the study area (see above). Save a few intact bodies, most samples were represented by prey fragments. Therefore, the minimum number of individuals of each prey type was calculated by counting body parts (Carlisle & Holberton 2006, Orłowski & Karg 2013).

In order to assess the possible spatial segregation of the study species, we performed point counts (Gibbons & Gregory 2006) at 72 stations in 2013 (11–17 April) and 58 in 2014 (16 and18 April). Counting stations were chosen from across the entire marshland by systematic selection. Each station was surveyed one time, the census lasted 10 minutes per station and the distance between each detected bird and the observer was recorded.

Statistical analysis

We calculated weekly food availability with the weighed abundance index (WAI) used by Poulin & Lefebvre (1997):

$$\mathrm{WAI} = \sum_{i=1}^{n} p_i \frac{x_{ij}}{y_i}$$

where p_i is the proportion of prey group (order) i in the birds' diet; x_{ij} is the number of prey of group i sweep-netted on date j; and y_i is the number of prey from group i sweep-netted during the whole sampling period. Birds' food sampling was not carried out weekly, unlike sweep-netting. Thus the x_{ij} of each week was associated with the p_i obtained from the chronologically closer food sampling.

With the diet composition and invertebrate sampling data, we assessed prey selection using Jacobs' index of selectivity (S; Jacobs 1974). It was calculated as $S = r - p/(r + p - 2rp)$, where r is the proportion of a prey type in diet and p is the proportion of that prey type in the environment. S begins at −1 (prey available in the environment, but never consumed) and comes close to 1 (the proportion of a given prey type in diet is far larger than in the environment). We tested the independence between prey availability and consumption using Fisher's exact test. To avoid proliferation of categories, we excluded the prey taxa sampled extremely rarely in the environment from this analysis (<5 individuals in the entire study period). To measure diet overlap, we calculated Pianka's overlap index (1973) using package pgirmess 1.5.9 (Giradoux 2014), in R 3.1.1 (R Core Team 2014). This index ranges between 0 (totally different diets) and 1 (identical diets). We also measured diet specialization with the Berger–Parker index of dominance (d), calculated as $d = N_{\max}/N$, where $N_{\max}$ is the number of individuals of the most abundant prey type; N is the total number of individuals in the sample (Berger & Parker 1970). Prey selection, niche overlap and diet specialization were calculated for the entire study period and per food sampling period.

Radial distances from the point transects were used to estimate density using program Distance 6.0 (Thomas *et al.* 2009). There were no observations closer than 5 m, thus zero distance was set at that point. This was probably due to the presence of the observer in the habitat. Data were right-truncated at a maximum distance of 50 m for Moustached Warblers and of 60 m for Reed Warblers. Data were grouped into 10 m wide intervals. In order to estimate the detection function, the half normal, uniform and hazard rate keys were used with the cosine, simple polynomial and hermite polynomial as series adjustments. The model that best fitted the data was selected using the Akaike Information Criteria (AIC). The model with the lowest AIC was chosen to obtain estimates (Burnham & Anderson 2002). The detection probability was estimated with all the combined data, while density and encounter rates were estimated per sample. The pooled estimate of density was made from the sample estimates treated as replicates. Afterwards we used Spearman rank correlation to assess the possible association between the densities of both species. The points without contacts were excluded from the analysis.

Results

Diet composition, prey selection and food niche overlap

The diet of both species exclusively included arthropods, which belonged to the orders *Araneida*, *Coleoptera*, *Diptera*, *Hemiptera*, *Hymenoptera* and (only in Reed Warblers' diet) *Orthoptera* (Table 1). *Coleoptera* and *Hymenoptera* were the most widely consumed prey and jointly represented 76.6% of Moustached Warblers' diet and 74.4% of Reed Warblers' diet. By studying prey choice over the entire study period, we found a clear selection for almost all prey types, and also similarly between both warbler species (Figure 1). In fact both species positively selected *Coleoptera*, *Araneida* and *Hymenoptera*, and negatively selected *Hemiptera* and *Diptera.* In all these cases, the difference between the proportion of prey type in the diet and in the environment was significant. When considering the single food sampling periods, prey preferences were less pronounced and not significant in some cases (Figure 1), possibly also because of the smaller sample size, but were still similar between both species. Eight other invertebrate categories (*Gasteropoda*, *Glomerida*, *Lepidoptera*, *Odonata*, *Parasitiformes*, *Thysanoptera*, Larvae and Eggs) were sampled in the environment, but were not encountered in food samples. In such cases, S always corresponds to −1. However, the proportions of these categories in the diet and in the environment were never significantly different. Thus their absence in food samples cannot be certainly ascribed to negative selection. According to the Pianka index, the trophic niche overlap was very large during all the food sampling periods (April = 0.934; May/June = 0.984; July = 0.995), and also during the whole study period (0.998). Diet specialization calculated over the entire data set was similar between the species (Moustached Warblers: $d = 0.387$; Reed Warblers: $d = 0.384$), but diverged slightly throughout the breeding season (Figure 2). For both species, specialization reached its

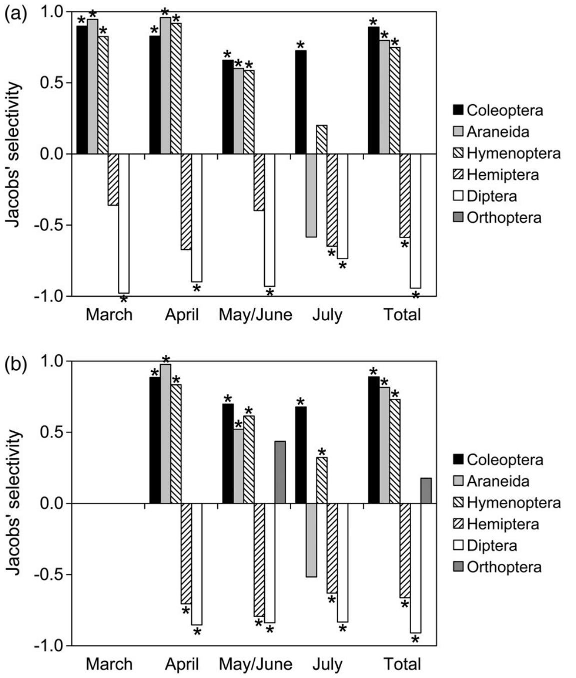

Figure 1. Prey selection of (a) Moustached and (b) Reed Warblers during the 2012 breeding season according to Jacob's index of selectivity (S). Asterisks mark the cases when the proportion of a prey type in diet and in the environment significantly differed according to Fisher's exact test. Only the prey types represented in diet are shown; for the other categories $S = -1$ and Fisher's exact test was never significant.

Table 1. Number of individuals and relative frequencies of the prey types in the food samples of Moustached ($N = 44$) and Reed ($N = 48$) Warblers collected during the 2012 breeding season. For Reed Warblers, we did not collect samples in March because this species was absent from the study area.

	Moustached Warblers					Reed Warblers			
Prey type	March	April	May–June	July	Total	April	May–June	July	Total
Araneida	6 (0.25)	2 (0.15)	5 (0.14)	1 (0.02)	14 (0.11)	18 (0.25)	7 (0.12)	2 (0.02)	27 (0.12)
Coleoptera	4 (0.17)	2 (0.15)	10 (0.29)	32 (0.62)	48 (0.39)	16 (0.22)	19 (0.32)	49 (0.57)	84 (0.38)
Diptera	1 (0.04)	1 (0.08)	1 (0.03)	2 (0.04)	5 (0.04)	8 (0.11)	4 (0.07)	2 (0.02)	14 (0.06)
Hemiptera	1 (0.04)	1 (0.08)	4 (0.11)	4 (0.08)	10 (0.08)	5 (0.07)	2 (0.03)	7 (0.08)	14 (0.06)
Hymenoptera	12 (0.50)	7 (0.54)	15 (0.43)	13 (0.25)	47 (0.38)	26 (0.36)	27 (0.45)	26 (0.30)	79 (0.36)
Orthoptera	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.02)	0 (0.00)	1 (0.005)
Total	24 (1.00)	13 (1.00)	35 (1.00)	52 (1.00)	124 (1.00)	73 (1.00)	60 (1.00)	86 (1.00)	219 (1.00)

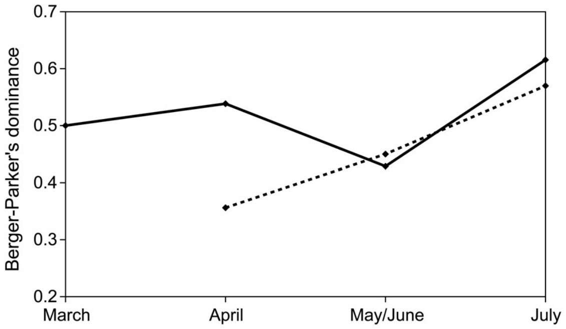

Figure 2. Diet specialization of Moustached (solid line) and Reed (dashed line) Warblers during the 2012 breeding season according to the Berger–Parker index of dominance (*d*).

maximum in July because of the large proportion of *Coleoptera* found in diets.

Temporal patterns of food availability and food demand

The breeding phenology data (Figure 3) allowed us to measure the temporal mismatch of the reproduction stages between the study species: the first incubating female was captured 7 weeks earlier for Moustached Warblers than for Reed Warblers and the first capture of a fledged young occurred 6 weeks earlier for Moustached Warblers than for Reed Warblers. The highest food demand period (nestlings rearing, see Methods) was estimated to be April for Moustached Warblers and approximately 5–25 June for Reed Warblers (Figure 4). As a result of this temporal difference, when adult Reed Warblers reared nestlings, both adults and an increasing number of young Moustached Warblers foraged in the reedbed. Furthermore, the higher food demand of Reed Warblers coincided with a second lower food demand peak of Moustached Warblers (Figure 4) due to the latter species' substitution/second clutches. Food availability, estimated with the WAI, was similar between both species (which is not surprising given the similar diets), and increased with fluctuations throughout the breeding season (Figure 4). For both species, the greatest food abundance was reached late in June. For Moustached Warblers, the highest food demand occurred when food availability rose (WAI range = 0.036–0.086), but was still below the maxima reached late in June (WAI = 0.106) and at the beginning of July (WAI = 0.100). The overall abundance of the potential competitors calculated for the corresponding weeks (6–10, Figure 5) ranged between 2.5 and 4.4 individuals per day (MA, see Methods), which was lower than for the following months, but still rapidly increased (Figure 5). For Reed Warblers, the WAI ranged between 0.045 and 0.104 during the highest food demand period, including greatest recorded availability, but the abundance of potential competitors was also high (MA range = 4.9–6.1 ind./day; Figure 5).

Spatial segregation

During the census of 2013, we recorded Moustached Warblers at 40 points, Reed Warblers at 33 points and the two species overlapped at 12 points. Thus 30.0% of Moustached Warbler occurrences overlapped spatially with Reed Warblers, and vice versa in 36.4% of cases. In 2014, we recorded Moustached Warblers at 20 points, Reed Warblers at 23 points and the two species overlapped at 9 points; that is, in 45.0% and 39.1% of the cases, respectively. A significant negative association between the densities of the two species

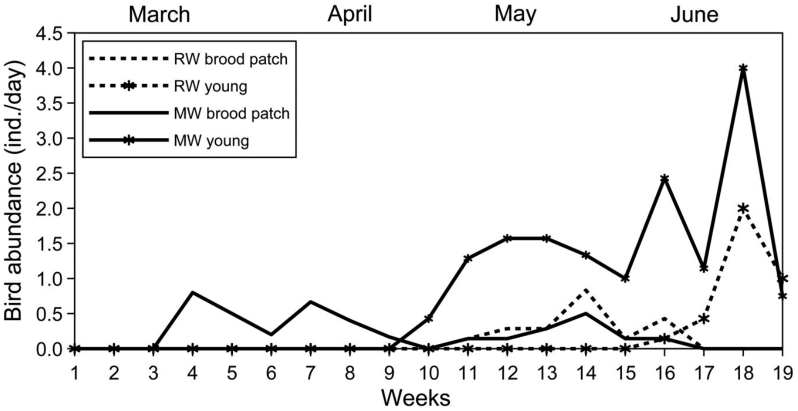

Figure 3. Weekly abundance of females with vascularized brood patches and young individuals of Moustached (MW) and Reed (RW) Warblers during the 2012 breeding season.

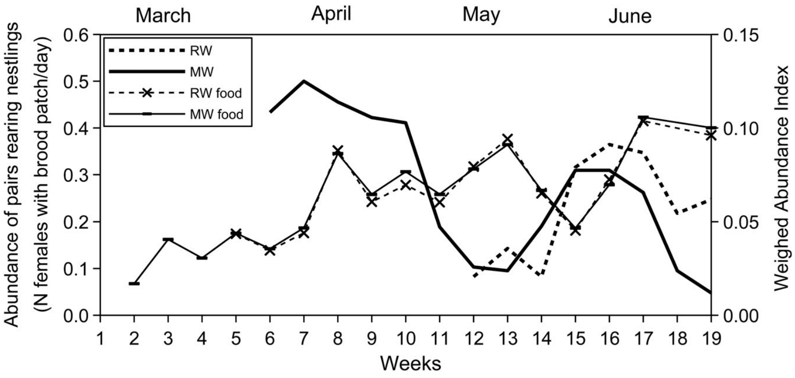

Figure 4. Abundance estimation of breeding pairs rearing nestlings of Moustached (MW) and Reed (RW) Warblers during the 2012 breeding season, calculated as the averaged abundance of females with evidently vascularized brood patches recorded in the previous 3 weeks, and food availability for the two species according to the WAI.

was found in 2013 but not 2014 (2013: rho = −0.635, $P < 0.001$; 2014: rho = −0.321, $P = 0.069$; Figure 6).

Discussion

Our results indicate a large trophic niche overlap and similar prey choice between the Moustached and Reed Warblers. These findings confirm that the two species are potential competitors for food. The low sample size should be taken into account when considering these results, especially for the single food sampling periods. The negative association between densities of Moustached and Reed Warblers in 2013 and the degree of spatial overlap (<50%) indicate a clear difference in the spatial distribution of both species in our study area. Given the greater habitat specialization of Moustached Warblers (see the references in the first section), its occurrence in only one part of the census points occupied by Reed Warblers is not surprising. Yet Reed Warblers were also contacted only in one part (<40%) of the Moustached Warblers' occurrence points. Reed Warblers are an *Acrocephalus* with eclectic habitat requirements (see the first section). Thus its absence from most areas occupied by Moustached Warblers, and consequently from wide reedbed areas (suitable habitat), needs explaining. We also observed a partial temporal segregation of breeding between both species, and the most important periods for the chick rearing of Moustached and Reed Warblers did not overlap. Thus, as predicted, we found evidence of niche differentiation among two potentially competing species.

Given these elements, we suggest that a small spatial and temporal overlap may be a response to interspecific competition for food. According to this scenario, Moustached Warblers reduced competition by breeding early, and partly avoided the higher food demand period of Reed Warblers. As a further possible advantage, the early fledged juveniles of Moustached Warblers did not have to compete with young Reed Warblers for several

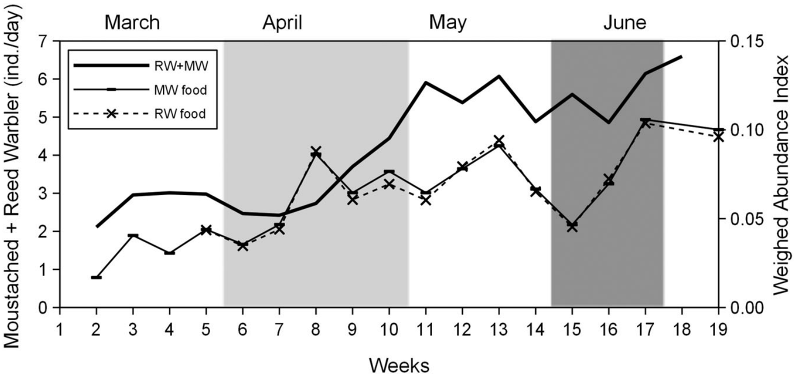

Figure 5. Weekly overall abundance of Moustached and Reed Warblers (RW + MW) during the 2012 breeding season (the solid line represents MA over 3 weeks), higher food demand periods of Moustached and Reed Warblers (light grey area and dark grey area, respectively) identified on the basis of brood patch data, and food availability for the two species according to the WAI.

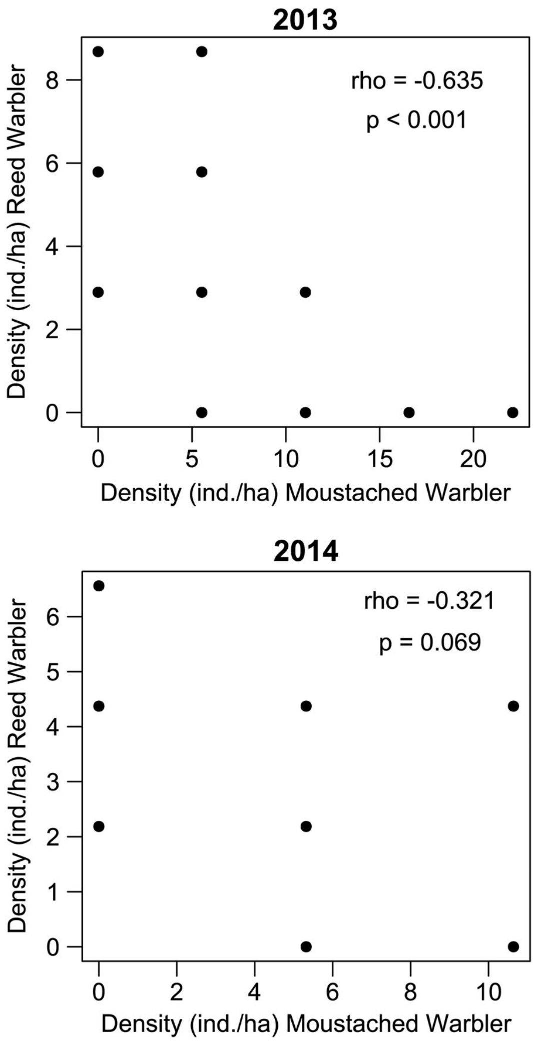

Figure 6. Associations between the densities of Moustached and Reed Warblers recorded at point count locations during the breeding seasons of 2013 (*N* points = 72) and 2014 (*N* points = 58).

weeks (Figure 3, weeks 10–15). In addition, early breeding implies the possibility to carry out a substitution/second clutch (Figure 4). The breeding phenology of Moustached Warblers varies notably among years and breeding sites, thus it is likely to be influenced by inter-annual climate variability and local factors (Castany & Lopez 2006 and references therein). The advantages that stem from lower competition may also influence the breeding phenology of Moustached Warblers and help explain the early breeding of this species. Concerning Reed Warblers, individuals arriving from Africa would be advantaged from settling preferably in those areas not previously occupied by Moustached Warblers, because they would experience less competition for food during nestling rearing.

A large trophic niche overlap does not necessarily imply interspecific competition, and no direct evidence was obtained that food availability was scarce compared to the demand observed during the study period, or at least part of it. An appropriate way to assess the occurrence of interspecific competition among our study species would be to compare their niche dimensions in breeding sites of sympatry and allopatry (Begon *et al.* 2006). For example, detailed data about habitat preferences of both species in our study area and in allopatry sites may have allowed us to distinguish the effects of their co-occurrence from those of the habitat features on the observed spatial patterns. Conversely, starting from our data it is possible to draw conclusions only about niche overlap. According to our results, it is possible to identify periods when competition was more likely to occur based on high food demand and fluctuations in food availability (see weeks 15–16, Figures 4 & 5). As a result of inter-annual climate variability, such fluctuations are likely to occur not only during one breeding season, but also between years, with competition being more likely in years with lower food availability. However, competition for food was unlikely to occur if food availability at our study site was higher than the overall demand throughout the breeding season. After studying several wetlands in south France, Poulin *et al.* (2002) found a significant positive correlation between the abundance of breeding reedbed-nesting passerines and food (arthropods) availability. This result does not support the scenario where food in Mediterranean reedbeds is generally overabundant compared with the demand of reedbed-nesting passerines because, in this case, food availability should not influence bird abundance. In other European regions, researchers who studied *Acrocephalus* warblers found no clear influence of food abundance on breeding success (Bibby & Thomas 1985) or attributed low importance to this factor in territorial spacing (Hoi *et al.* 1991). Other researchers who studied these species found possible signs of competition: Castany (2003) reported a surprisingly low breeding density of Reed Warblers in a Spanish marshland that hosts a large population of Moustached Warblers. This author hypothesized that such a low density could be explained by the competition of both Moustached and Great Reed Warblers; Castany (2003) and Poulin *et al.* (2002) reported Reed Warblers to be common in areas that are not optimal for other species, such as dry reedbed zones. Thus this species' eclectic habitat requirements may enable it to occupy the reedbed areas that other species avoid, which would reduce interspecific competition.

Factors other than interspecific competition for food may explain the segregation we observed. Some authors have reported interspecific aggressive interactions (interference competition, Begon *et al.* 2006) among *Acrocephalus* warblers, but the meaning and effects of such behaviours have not been clarified (Murray 1971, Catchpole 1978, Hoi *et al.* 1991). In a Central Europe marshland, Moustached Warblers bred in a limited area within the wider spatial range of Reed Warblers, and no dominant relationship between the two species was found (Hoi *et al.* 1991). While conducting fieldwork in areas where the two species co-occur, we noticed no interactions to support the existence of interference competition. However, we did not carry out specific observations or experiments to study aggressive interactions. While studying *Phylloscopus* warblers, Bourski & Forstmeier (2000) suggested that some species may simply avoid areas occupied by the congeneric competitors that arrived earlier. Given the apparent lack of aggressive interactions, they also suggested the term 'territorial avoidance' as being more correct than 'territorialism'.

A further possible explanation has been suggested by Hoi *et al.* (1991): territorial spacing and differences in the breeding times between *Acrocephalus* warblers may operate to reduce nest predation. As we did not collect data on this issue, we were unable to evaluate this possibility in our study.

By way of conclusion, we found a large trophic niche overlap at our study site between Moustached and Reed Warblers throughout the breeding season. Conversely, we found differences in space and time use between both species. We suggest that these differences may be a response to interspecific competition for food. The need to reduce competition may also help explain the early breeding of Moustached Warblers. Although we found no direct evidence for the occurrence of competition, we identified periods when interspecific competition was more likely to occur given high food demand and fluctuations in food availability. Other factors, such as interspecific aggressive interactions and nest predation, may also help determine the observed spatial and temporal patterns.

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References

Ausden, M. & Drake, M. 2006. Invertebrates. In Sutherland, W.J. (eds.) *Ecological Census Techniques: A Handbook*, 214–249. Cambridge University Press, Cambridge.

Bailey, R.E. 1952. The incubation patch of passerine birds. *Condor* **54:** 121–136.

Barrientos, J.A. 2004. *Curso práctico de entomología*. Universitat Autonòma de Barcelona, Barcelona.

Begon, M., Townsend, C.R. & Harper, J.L. 2006. *Ecology: From Individual to Ecosystems*, 4th edn. Blackwell Publishing, Oxford.

Berger, W.H. & Parker, F.L. 1970. Diversity of planktonic foraminifera in deep sea sediments. *Science* **168:** 1345–1347.

Bibby, C.J. & Thomas, D.K. 1985. Breeding and diets of the Reed Warbler at a rich and a poor site. *Bird Study* **32:** 19–31.

Bourski, O.V. & Forstmeier, W. 2000. Does interspecific competition affect territorial distribution of birds? A long-term study on Siberian *Phylloscopus* warblers. *Oikos* **88:** 341–350.

Burnham, K.P. & Anderson, D.R. 2002. *Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach*. Springer Verlag, New York.

Carlisle, J.D. & Holberton, R.L. 2006. Relative efficiency of fecal versus regurgitated samples for assessing diet and the deleterious effects of a tartar on migratory birds. *J. Field Ornithol.* **77:** 126–135.

Castany, J. 2003. El carricerín real (*Acrocephalus melanopogon*) en el P. N. del Prat de Cabanes-Torreblanca. Doctoral Thesis. University of Valencia.

Castany, J. & Lopez, G. 2006. *El carricerín real en España. I Censo Nacional (2005)*. SEO/Birdlife, Madrid.

Catchpole, C.K. 1978. Interspecific territorialism and competition in *Acrocephalus* warblers as revealed by playback experiments in areas of sympatry and allopatry. *Anim. Behav.* **26:** 1072–1080.

Ceresa, F., Belda, E.J. & Monrós, J.S. 2014. Apomorphine as an emetic for insectivorous songbirds: effectiveness and post-release effects on survival and mass change. *J. Field Ornithol.* **85:** 213–220.

Clarabuch, O. 2000. El estudio del ave en mano. In Pinilla, J. (ed.) *Manual para el anillamiento científico de aves*, 73–98. SEO/BirdLife, Madrid.

Gibbons, D.W. & Gregory, R.D. 2006. Birds. In Sutherland, W.J. (eds.) *Ecological Census Techniques: A Handbook*, 308–350. Cambridge University Press, Cambridge.

Giradoux, P. 2014. Package 'pgirmess' 1.5.9. Data analysis in ecology. http://cran.r-project.org/web/packages/pgirmess/index.html

Gonzalez-Solis, J., Oro, D., Jover, L., Ruiz, X. & Pedrocchi, V. 1997. Trophic niche width and overlap of two sympatric gulls in the southwestern mediterranean. *Oecologia* **112:** 75–80.

Hoi, H., Eichler, T. & Dittami, J. 1991. Territorial spacing and interspecific competition in three species of reed warblers. *Oecologia* **87:** 443–448.

Honza, M., Moksnes, A., Roskraft, E. & Øien, I.J. 1999. Effect of great reed warbler *Acrocephalus arundinaceus* on the reproductive tactics of the reed warbler *A. scirpaceus*. *Ibis* **141:** 489–493.

Jacobs, J. 1974. Quantitative measurements of food selection: a modification of the forage ratio and Ivlev's electivity index. *Oecologia* **14:** 413–417.

Kennerley, P. & Pearson, D. 2010. *Reed and Bush Warblers*. Christopher Helm Publishers Ltd, London.

Lack, D. 1968. *Ecological Adaptation for Breeding in Birds*. Methuen, London.

Morelli, F. & Tryjanowski, P. 2015. No species is an island: testing the effects of biotic interactions on models of avian niche occupation. *Ecol. Evol.* **5:** 759–768.

Murray, B.G. 1971. The ecological consequences of interspecific territorial bahavior in birds. *Ecology* **52:** 414–423.

van Noordwijk, A.J., McCleery, R.H. & Perrins, C.M. 1995. Selection for the timing of great tit (*Parus major*) breeding in relation to caterpillar growth and temperature. *J. Anim. Ecol.* **64:** 451–458.

Orłowski, G. & Karg, J. 2013. Diet breadth and overlap in three sympatric aerial insectivorous birds at the same location. *Bird Study* **60:** 475–483.

Perrins, C.M. & McCleery, R.H. 1989. Laying dates and clutch size in the Great Tit. *Wilson Bull.* **101:** 236–253.

Pianka, E.G. 1973. The structure of lizard communities. *Annu. Rev. Ecol. Syst.* **4:** 53–74.

Poulin, B. & Lefebvre, G. 1997. Estimation of arthropods available to birds: effect of trapping technique, prey distribution, and bird diet. *J. Field Ornithol.* **68:** 426–442.

Poulin, B., Lefebvre, G. & Mauchamp, A. 2002. Habitat requirements of passerines and reedbed management in southern France. *Biol. Conserv.* **107:** 315–325.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing http://www.R-project.org

Schoener, T.W. 1974. Resource partitioning in ecological communities. *Science* **185:** 27–39.

Shiel, C., McAney, C., Sullivan, C. & Fairley, J. 1997. *Identification of Arthropod Fragments in Bat Droppings*. Mammal Society, London.

Svensson, L. 1992. *Identification Guide to European Passerines*, 4th edn. Svensson, Stockholm.

Thomas, L., Laake, J.L., Rexstad, E., Strindberg, S., Marques, F.F.C., Buckland, S.T., Borchers, D.L., Anderson, D.R., Burnham, K.P., Burt, M.L., Hedley, S.L., Pollard, J.H., Bishop, J.R.B. & Marques, T.A. 2009. Distance 6.0. Release '2'. – Research Unit for Wildlife Population Assessment, University of St. Andrews, UK. http://ruwpa.st-and.ac.uk/distance/

Urios, V., Donat, P. & Viñals, M.J. 1993. *La marjal de Pego-Oliva*. Instituto de estudios Comarcales de la marina Alta, Valencia.

Vieira, E.M. & Port, D. 2007. Niche overlap and resource partitioning between two sympatric fox species in southern Brazil. *J. Zool.* **272:** 57–63.

Wiens, J.A. 1977. On competition and variable environments. *Am. Sci.* **65:** 590–597.

Chapter 3

Does fragmentation of wetlands affect gene flow in sympatric *Acrocephalus* warblers with different migration strategies?

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Does fragmentation of wetlands affect gene flow in sympatric *Acrocephalus* warblers with different migration strategies?

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Wetlands are naturally patchy habitats, but patchiness has been accentuated by the extensive wetlands loss due to human activities. In such a fragmented habitat, dispersal ability is especially important to maintain gene flow between populations. Here we studied population structure, genetic diversity and demographic history of Iberian and North African populations of two wetland passerines, the Eurasian reed warbler *Acrocephalus scirpaceus* and the moustached warbler *Acrocephalus melanopogon*. These species are closely related and sympatric in our study sites, but the reed warbler is a widespread long-distance migrant while the moustached warbler's breeding range is patchier and it is resident or migrates over short distances. Using microsatellite and mtDNA data, we found higher differentiation in moustached than in reed warblers, indicating higher dispersal capability of the latter species. Our results also suggest that the sea limits dispersal in the moustached warbler. However, we found evidence of gene flow between the study sites in both species, indicating a capability to compensate for habitat fragmentation. In most cases, the gene flow was restricted, possibly because of the large distances between study sites (from ca 290 to 960 km) or breeding site fidelity. The reed warbler had higher haplotype diversity, likely due to dispersal from different populations, past admixture event and a larger population size. We found also signs of postglacial population growth for both species and evidence of a recent colonization or re-colonization of the Mallorca Island by the moustached warbler.

Habitat fragmentation consists of reduction of the total habitat area and creation of separate patches from a wider continuous distribution (Frankham et al. 2010). The consequences of habitat fragmentation on the demographic and genetic structure of a natural population vary according to both landscape features (degree of habitat isolation, type of matrix between fragments) and species characteristics (population size and density, dispersal ability, stress tolerance; Matthysen et al. 1995, Newton 1998, Bohonak 1999, Desrochers et al. 1999, Galbusera et al. 2004). Wetlands are patchy habitats immersed in a terrestrial matrix, and this natural characteristic of discontinuity has been accentuated by the extensive habitat destruction caused by human activities (Finlayson et al. 1992, Van Vessem et al. 1997, Paracuellos and Tellería 2004, Silva et al. 2007, Laurence 2010). In such a scattered habitat, the dispersal ability of a species is crucial to produce sufficient gene flow to reduce the impact of population fragmentation. Lack of gene flow between fragmented populations can lead to loss of genetic diversity (Kvist et al. 2011), inbreeding and consequently higher extinction risk compared to a continuous population (Frankham et al. 2010). Dispersal ability of birds is generally high (Koenig et al. 1996, Frankham et al. 2010), but detailed species-specific estimates are difficult to obtain. This is largely due to practical difficulties; studying dispersal over large distances requires large-scale marking schemes (Paradis et al. 1998, Hansson et al. 2002) and the use of satellite telemetry is usually possible only on a reduced number of individuals due to the high costs and cannot be used in small birds due to their size. However, indirect genetic methods can provide useful information about gene flow and population differentiation.

Here we present new information of genetic diversity and population structure of two closely related and sympatric wetland passerines with different migration strategies, the moustached warbler *Acrocephalus melanopogon* and the Eurasian reed warbler *Acrocephalus scirpaceus* (hereafter reed warbler), based on both microsatellites and mitochondrial DNA data.

The moustached warbler is a polytypic species breeding across a discontinuous area ranging from SW Europe to central Asia (Kennerley and Pearson 2010). Our study populations in Spain belong to the nominal subspecies *melanopogon*, which occupy the western part of the species range. These populations are mostly sedentary or migrate over short distances (Castany 2003, Castany and López 2006, Kennerley and Pearson 2010). In Spain, the distribution of the moustached warbler is discontinuous and most of the ~ 1000 breeding pairs are concentrated in a few marshlands along the Mediterranean Coast and on the Mallorca Island (Castany and López 2006). Until now, no information about genetic diversity and genetic population structure has been provided for this species.

The reed warbler is a common and widespread breeding bird in Europe. This species is a long-distance migrant wintering in sub-Saharan Africa (Kennerley and Pearson 2010); a migratory divide in central Europe splits the European reed warbler population (subspecies *scirpaceus*) into SW- and SE-migrating populations (Procházka et al. 2008). Iberian reed warblers belong to the SW-migrating group, have more rounded and shorter wings (Cramp 1992, Peiró 2003) and winter more north than the other SW-migrating populations (Procházka et al. 2008). Procházka et al. (2011), using ten microsatellite loci, found no clear population structure and low genetic differentiation in reed warbler populations across Europe, indicating a high level of gene flow. Furthermore, the authors reported slight, but significant, differentiation of Iberian populations and suggested that they may have a different evolutionary history than other populations. North African reed warblers seem to be partly sedentary (Amezian et al. 2010, Kennerley and Pearson 2010) and birds breeding in Morocco differ from European reed warblers also in biometrics, coloration and moult strategy (Amezian et al. 2010, Jiguet et al. 2010). On this basis, some authors suggest that these birds could constitute a new taxon (Amezian et al. 2010, Jiguet et al. 2010), thus the status of the north African population is still unclear.

The aims of this study were: 1) to provide new information of genetic diversity, population structure and population history of the study species in Iberia (and also in north Africa for the reed warbler), 2) to assess the possible effects of habitat fragmentation on the genetic diversity and structure of the study populations and 3) to compare the results obtained for the two species, taking into account especially their differences in migration strategy and population size.

Methods

Sampling and DNA extraction

We obtained blood samples from birds mist-netted during the breeding season of 2012–2013 at four study areas: the Pego-Oliva Natural Park (38°51′N, 0°03′W), S'Albufera de Mallorca (39°47′N, 3°06′E) and Villafranca de los Caballeros (39°27′N, 3°19′W) in Spain and Larache (35°08′N, 6°05′W) in Morocco (Fig. 1, for sample sizes see Table 1). At the last site, the breeding population of the moustached warbler is small and we captured no individuals. Blood samples (5–15 µl) were obtained by puncturing the brachial vein and stored in 96% ethanol. Overall, we sampled 54 moustached warblers and 68 reed warblers. We extracted DNA using UltraClean™ Blood Spin™ Kit (MoBio Laboratories) according to the manufacturer's protocol.

DNA amplification

Microsatellites

We amplified 16 polymorphic microsatellite loci from reed warbler DNA samples: Ase25, Ase34, Ase37, Ase48, Ase58 (Richardson et al. 2000, *Acrocephalus sechellensis*), Pocc2 (Bensch et al. 1997, *Phylloscopus occipitalis*), Ppi2 (Martínez et al. 1999, *Pica pica*), Aar4, Aar5 (Hansson et al. 2000, *Acrocephalus arundinaceus*), FhU2 (Ellegren 1992, *Ficedula hypoleuca*), Pca3 (Dawson et al. 2000, *Cyanistes caeruleus*), Pdoµ1 (Neumann and Wetton 1996, *Passer domesticus*), Cuµ28 (Gibbs et al. 1999, *Catharus ustulatus*), Gf05 (Petren 1998, *Geospiza fortis*), Pdo5 (Griffith et al. 1999, *P. domesticus*) and ZL54 (Frentiu et al. 2003, *Zosterops lateralis*). From the moustached warbler, we successfully amplified eight polymorphic loci: Ase18 (Richardson et al. 2000, *A. sechellensis*), Aar4, Pdo5, Ppi2, ZL54, Pocc2, Pca3 and Ase25. Details on the PCR are available in Supplementary material Appendix 1. We ran the PCR products with ABI PRISM 3730 DNA Analyzer (Applied Biosystems) and scored them with GeneMapper 4.0. We checked the data with the program MICROCHECKER 2.2.3 (van Oosterhout et al. 2004) for possible genotyping errors (null alleles, scoring errors due to stuttering, large allele dropouts).

Mitochondrial DNA

We amplified 473 bp (reed warblers) of the mitochondrial cytochrome oxidase COI using primers CO1F and CO1R (Hebert et al. 2004) or 623 bp (moustached warbler) using primers CO1F and CO1R2 (Kerr et al. 2007). Details on the PCR

Figure 1. Location of the sampling sites: 1) Larache, 2) Villafranca de los Caballeros, 3) Pego-Oliva Natural Park and 4) S'Albufera de Mallorca.

Table 1. Sample sizes, expected heterozygosity (H_e), allelic richness (A), inbreeding coefficient (F_{IS}), Wilcoxon p-values (Wil. test, one-tailed for heterozygosity excess) and Garza–Williamson index (G–W) of (a) reed warbler and (b) moustached warbler, calculated from microsatellite data for each sampling site and populations defined by STRUCTURE (Str).

Sample	n	H_e (SD)	A (SD)	F_{IS} (SD)	p (Wil. test)	G–W
(a)						
Larache	38	0.696 (0.316)	1.70 (0.32)	0.127 (0.314)	0.207	0.84
Pego-Oliva	7	0.681 (0.366)	1.68 (0.37)	0.049 (0.281)		0.67
Mallorca	3	0.650 (0.375)	1.65 (0.38)	–0.153 (0.413)		0.62
Villafranca	20	0.710 (0.249)	1.71 (0.25)	–0.022 (0.257)	0.661	0.73
Total	68	0.716 (0.272)	1.68	0.014 (0.316)		1.00
(b)						
Pego-Oliva	30	0.602 (0.184)	3.76 (2.16)	–0.275 (0.404)	0.078	0.96
Villafranca	9	0.590 (0.185)	3.47 (2.22)	–0.344 (0.477)	0.078	0.85
Mallorca (= Str – Mallorca)	15	0.538 (0.202)	2.96 (1.60)	–0.500 (0.454)	0.023	0.82
Str – Spanish mainland	39	0.611 (0.172)	4.28 (3.01)	–0.240 (0.394)	0.039	0.96
Total	54	0.621 (0.182)	3.40	–0.250 (0.115)		0.88

procedures are available in the online supplementary material. We used the forward primer (F) for sequencing and, in order to check for sequence quality, all the reed warblers and 26 moustached warblers were sequenced also using the reverse primers (R). We used the BigDye™ Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems) and ran the sequencing reactions with ABI PRISM 3730 automatic sequencer (Applied Biosystems). We obtained sequences of 50 reed warblers and 43 moustached warblers (GenBank accession numbers: KP776472 to KP776521 for the first species and KP776522 to KP776564 for the second species). The sequences were checked and aligned with BioEdit 7.2.5 (Hall 1999).

Statistical analysis

Microsatellites

We tested the possible deviations from the Hardy–Weinberg and linkage equilibrium with GENEPOP 4.2 (Raymond and Rousset 1995, Rousset 2008), for each sampling site and for the total sample of both species. For the same groups we calculated the expected heterozygosity (H_e) with Arlequin 3.5.1 (Excoffier and Lischer 2010) and inbreeding coefficient (F_{IS}) and allelic richness (A) with FSTAT 2.9.3 (Goudet 1995).

To infer the population genetic structure we used the program STRUCTURE 2.3.4 (Pritchard et al. 2000, Falush et al. 2003). This program is based on a Bayesian approach and allows estimation of the most probable number of distinct genetic clusters (K) in the data set. We chose a model with population admixture and correlated allele frequencies (Falush et al. 2003), and performed the analysis with and without prior spatial information of sample locations. We performed ten independent runs for each value of K between 1 and 10, with a burn-in period of 50 000 iterations and 500 000 Markov chain Monte Carlo (MCMC) replications. Furthermore, we calculated the ad hoc statistic ΔK from the STRUCTURE results as described by Evanno et al. (2005). We investigated population differentiation also by calculating pairwise F_{ST} values between each of the sampling sites using the program Arlequin.

We explored the spatial genetic structure with the program SPAGeDi 1.4 (Hardy and Vekemans 2002), using the Loiselle kinship coefficient (Loiselle et al. 1995) and four (reed warbler) or three (moustached warbler) distance classes. The spatial coordinates of the individuals corresponded to the coordinates of the four sampling sites, and we built distance classes to include one site per class. To obtain information about current dispersal between populations, we carried out an assignment analysis and looked for first generation migrants with the program GENECLASS 2 (Piry et al. 2004). We used the Bayesian individual assignment methods by Rannala and Mountain (1997) and the simulation algorithm of Paetkau et al. (2004). For both assignment analysis and first generation migrant detection, we used 1000 replicates, alpha level for the MCMC simulations at 0.01 and assignment threshold at 0.05.

Genetic data from population samples carry also information about population history. First, we looked for past bottlenecks by means of the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996, Piry et al. 1999), which tests for heterozygosity excess caused by recent reduction of the effective population size (Piry et al. 1999). We used the Wilcoxon test under the two-phase mutation model with 95% single-step mutations. We also estimated the Garza–Williamson index (Garza and Williamson 2001) with Arlequin, in order to search for strong past population bottlenecks. Using the genetic clusters identified by STRUCTURE, we studied the population history with the program DIY ABC 2.0.3 (Cornuet et al. 2008), based on approximate Bayesian computation (ABC). Using DIY ABC it is possible to compare different competing historical/demographic scenarios and to obtain parameter estimators, such as the divergence times and the current and past effective population sizes. For the reed warblers, we found only one genetic cluster with STRUCTURE (K = 1, see Results), thus we performed this analysis only for moustached warbler (K = 2, see Results). We explored five scenarios, the simplest one containing one divergence event, while the remaining four contained population size changes at different times after divergence in only one or both diverged lineages. We chose the default range of priors for effective population sizes and divergence times (10–10 000) and set the conditions for the chronological order of historical events. We adopted the default generalized stepwise mutation model (Estoup et al. 2002) and seven of 11 default summary statistics (four within- and three among-populations). With these settings, a total of 5 000 000 simulated data sets were calculated (1 000 000 per scenario); among them, the 50 000 sets closest to the observed data according to the summary statistics were used for parameter estimation.

Mitochondrial DNA

We calculated haplotype and nucleotide diversities for the entire sample and for each sampling site using DnaSP 5.10.01 (Librado and Rozas 2009). To build a statistical parsimony network, we used the program TCS 1.21 (Clement et al. 2000) with default settings of 95% parsimony connection limit. As outgroups, we included sequences from other European populations obtained from the GenBank (accession numbers and origin: moustached warbler – GQ481257, Russia; reed warbler – GU571698, Sweden). We used the program Arlequin to estimate population differentiation by calculating pairwise φ_{ST} values between each of the sampling sites using pairwise differences and frequencies of haplotypes.

We studied the demographic history of both species with mismatch distribution analysis, the distributions of the observed number of differences between pairs of sequences in a sample. Unimodal distributions usually indicate an expansion event, whereas multimodal distributions are typical of populations at demographic equilibrium (Rogers and Harpending 1992). In addition, we carried out Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) neutrality tests, where a statistically significant negative value indicates a recent expansion event. All these were tested for the entire sample and for each sampling site separately using Arlequin and DnaSP.

The reed warbler mismatch distribution suggested an admixture of two previously isolated populations (see Results). Thus, we ran reed warbler data with DIY ABC to estimate the timing of this demographic event. We compared four scenarios, where the simplest one consisted of an admixture of lineages at time t_a and a previous divergence at time t_2. The remaining three scenarios followed the same basic setting, but included population size changes at different times for only one or both lineages before the admixture. Reed warbler populations in Iberia and across Europe are abundant (Carrascal and Palomino 2008, BirdLife International 2012), thus we set the maximum effective population size at 1 000 000 individuals in the model. As maximum time for the admixture and the previous divergence events, we set respectively 10 000 and 20 000 generations. We used the Kimura two parameter mutation model (Kimura 1980) and all default summary statistics. A total of 4 000 000 simulated data sets were calculated (1 000 000 per scenario); among them, the 40 000 sets closest to the observed data according to the summary statistics were used for parameter estimation.

Results

Reed warbler

Microsatellites

Using MICROCHECKER we found possible null alleles in loci Ase25, Ase37, Ase48 and ZL54. We excluded these loci from calculations for the Hardy–Weinberg equilibrium, linkage disequilibrium and inbreeding coefficient, as well as from BOTTLENECK analysis, while for the other analyses we used all 16 loci. We found no evidences of large allele dropouts or scoring errors in the data set.

We found no significant deviations from Hardy–Weinberg equilibrium. Linkage disequilibrium was found only for FhU2 and Pca3 in Villafranca and when all sampling sites were combined. Genetic variation (Table 1a) was at similar levels in all the four sampling sites, the highest was found in Villafranca ($H_e = 0.710$, $A = 1.71$) and the lowest in Mallorca ($H_e = 0.650$, $A = 1.65$).

The program STRUCTURE attributed the highest likelihood (–2412) to $K = 1$, although $K = 2$ obtained almost equal support (–2417). ΔK showed a peak at $K = 2$, but as it is not possible to compute ΔK for $K = 1$, we could not compare the two hypotheses based on this. The STRUCTURE bar plot obtained for $K = 2$ (Fig. 2a) support the $K = 1$ hypothesis, because all individuals showed approximately equal probability of belonging to both of the two clusters. To detect a possible weak population structuring we repeated the analysis adding geographical information (LOCPRIOR model), but with similar results. Therefore, the most supported hypothesis is the lack of a clear population structuring in reed warblers. Nonetheless, in two cases (Larache – Pego-Oliva and Villafranca – Mallorca) pairwise F_{ST} values between sampling sites were significant, though low (Table 2a).

Using the program SPAGeDi, the regression between the kinship coefficients of individual pairs and the logarithm of the distances between sampling sites was significantly negative ($r = –0.058$, $p = 0.042$). However, given the low regression coefficient, this does not clearly support isolation by distance. Out of 68 birds, 43 (63%) were assigned to their sampling sites (Table 3a) by the assignment test. It is noteworthy, that two birds sampled at Mallorca were identified as first generation migrants and assigned to Pego-Oliva. Both individuals (a male with evident cloacal protuberance and a female with brood patch) were breeding when sampled.

We found no evidence of past bottlenecks according to the allele frequency distributions or heterozygote excess (Table 1a). Sample sizes from Pego-Oliva and Mallorca were too small to perform the analysis. The Garza–Williamson (G–W) index provides a sign of past bottleneck when lower than 0.68 while excludes this event if it exceeds 0.8 (Garza and Williamson 2001). The only low values were obtained from Pego-Oliva and Mallorca (Table 1a), but again, the small sample sizes prevent further interpretations. To conclude, we found no clear evidence of past bottlenecks.

Mitochondrial DNA

The 50 sequenced reed warblers belonged to 18 different haplotypes. The TCS network (Fig. 3a) show a star-like structure, with one dominant haplotype including 25 individuals (50% of the total) and 17 other haplotypes represented by few birds (1–4). This structure suggests past population expansion. Four of sampled birds were more related to the outgroup individual from Sweden than to the remaining Iberian/African individuals (Fig. 3a). Haplotype and nucleotide diversities are reported in Table 4a. The pairwise φ_{ST} values between the study sites were low and not significant (Table 5a).

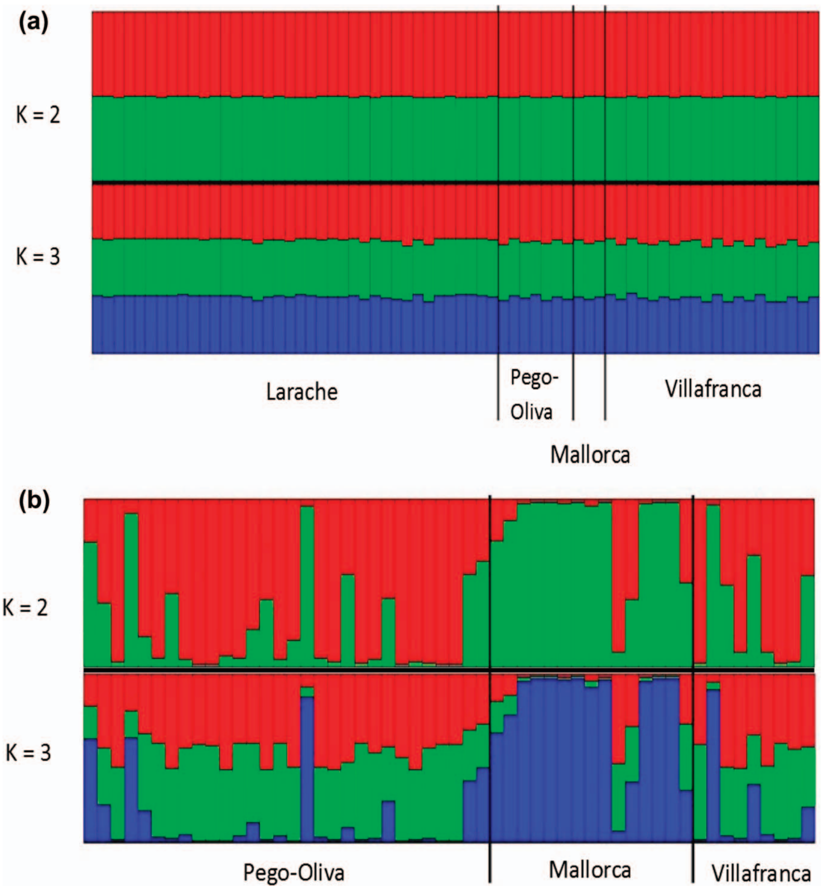

Figure 2. Bar plots of the STRUCTURE results based microsatellite data of (a) reed warbler and (b) moustached warbler for $K = 2$ and $K = 3$. Each column corresponds to an individual and represents its probability to belong to one of the K clusters.

According to the neutrality tests, we found signs of population expansion for Larache and, less clearly, for the total data set. Tajima's D and Fu's F_S were significantly negative for Larache ($D = -2.203$, $p < 0.01$; $F_S = -7.063$, $p = 0.001$) and for the total sample, only Fu's F_S was significantly negative ($D = -1.581$, $p > 0.05$; $F_S = -8.590$, $p < 0.001$). The mismatch distribution did not detect significant deviations from the expansion hypothesis. Furthermore, mismatch

Table 2. Pairwise F_{ST} values (p-values in parentheses) between sampling sites and between STRUCTURE (Str) populations of (a) reed warbler and (b) moustached warbler, calculated from microsatellite data.

Sample	Pego-Oliva	Mallorca	Villafranca
(a)			
Mallorca	0.0030 (0.189)	–	–
Villafranca	–0.0211 (0.505)	0.0732 (0.018)	–
Larache	0.0189 (0.027)	–0.0029 (0.351)	–0.0193 (0.892)

Sample	Pego-Oliva	Mallorca (= Str – Mallorca)
(b)		
Mallorca	0.1146 (0.000)	–
Villafranca	0.0609 (0.000)	0.1352 (0.000)
Str – Spanish mainland	–	0.1078 (0.000)

Table 3. Assignment of (a) reed warblers and (b) moustached warblers to the sampling sites and the genetic clusters defined by STRUCTURE (Str), on the basis of microsatellite data; individuals which have not been assigned to their site/population of origin are reported in bold.

	Assigned to			
Sampling site of origin	Larache	Pego-Oliva	Mallorca	Villafranca
(a)				
Larache	35	**1**	0	**2**
Pego-Oliva	**3**	0	0	**4**
Mallorca	**2**	0	0	**1**
Villafranca	**12**	0	0	8

	Assigned to		
Sampling site of origin	Pego-Oliva	Mallorca	Villafranca
(b)			
Pego-Oliva	30	0	0
Mallorca	**5**	9	**1**
Villafranca	**6**	0	3

	Assigned to	
Str population of origin	Str – Spanish mainland	Str – Mallorca
Str – Spanish mainland	39	0
Str – Mallorca	**6**	9

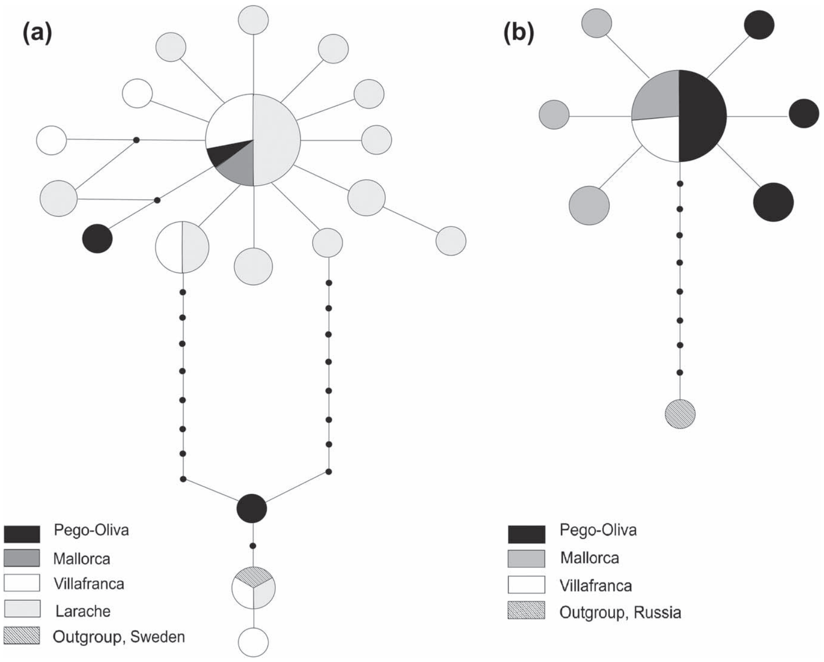

Figure 3. Statistical parsimony network based on (a) reed warbler and (b) moustached warbler mtDNA data. Each haplotype is represented by a circle, whose area is proportional to the number of individuals belonging to the haplotype. Each connecting bar stands for one substitution.

distribution of Larache, Villafranca and the whole data set showed a bimodal shape (Fig. 4), suggesting a past admixture of previously isolated populations. When demographic history was explored with DIY ABC, the scenario including a population size change in the other population after divergence but before admixture (hereafter A; Fig. 5) resulted to be the best. However, the simplest scenario with divergence followed by admixture without population size changes (hereafter B; Fig. 5), obtained high support as well, thus we report the parameter estimates from both scenarios (Fig. 5). Scenario A had lower posterior probability than B according to direct estimation (A = 0.386, B = 0.440), but the logistic approach clearly supported scenario A (A = 0.841, B = 0.159). Testing confidence in scenario choice over all scenarios, as implemented in DIY ABC, confirmed scenario A as the best, but with high type I error (56.8% according to direct approach, 55.4% to logistic approach) due to the good performance of scenario B. Both scenarios fitted the data well according to model checking, with no significant deviation in summary statistics between posterior distributions and observed data. Timing of admixture was estimated to be 5690 generations ago (CI 95% 583–9610) from scenario A and 4750 generations (CI 95% 505–9420) from scenario B. Assuming a generation length of three years (mean of ages suggested for other *Acrocephalus* warblers; Bensch and Hasselquist 1999, Spurgin et al. 2014), this would translate to admixture occurring about 14 000–17 000 yr ago.

Table 4. Sample sizes, number of segregating sites, number of haplotypes, haplotype diversity (h) and nucleotide diversity (π) of (a) reed warbler and (b) moustached warbler, calculated from mitochondrial DNA data for each sampling site and STRUCTURE (Str) population (Str populations were identified on the basis of microsatellite data).

Sample	n	n segregating sites	n haplotypes	h	π
(a)					
Larache	29	21	13	0.823	0.004
Pego-Oliva	5	11	3	0.700	0.010
Mallorca	3	0	1	0.000	0.000
Villafranca	13	14	6	0.718	0.008
Total	50	23	18	0.745	0.006
(b)					
Pego-Oliva	22	3	4	0.333	0.001
Villafranca	8	0	1	0.000	0.000
Mallorca (= Str – Mallorca)	13	4	4	0.526	0.001
Str – Spanish mainland	30	3	4	0.251	0.000
Total	43	7	7	0.339	0.001

Table 5. Pairwise φ_{ST} values (p-values in parentheses) between sampling sites and between STRUCTURE (Str) populations of (a) reed warbler and (b) moustached warbler, calculated from mitochondrial DNA data. STRUCTURE population were identified on the basis of microsatellite data.

Sample	Pego-Oliva	Mallorca	Villafranca
(a)			
Mallorca	–0.0918 (0.802)	–	–
Villafranca	–0.0997 (0.685)	–0.1149 (0.991)	–
Larache	0.0486 (0.207)	–0.1742 (0.991)	0.0302 (0.072)
Sample	Pego-Oliva	Mallorca (= Str – Mallorca)	
(b)			
Mallorca	0.0660 (0.036)	–	
Villafranca	–0.0402 (0.739)	0.0065 (0.541)	
Str – Spanish mainland	–	0.0862 (0.000)	

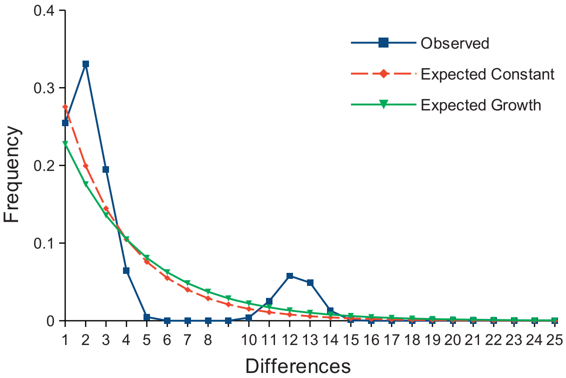

Figure 4. Mismatch distributions calculated from all reed warbler samples using mitochondrial DNA data. The bimodal shape of the observed frequencies indicates a past admixture of two previously isolated populations. Expected distributions for constant and expanded populations are also shown.

Moustached warbler

Microsatellites

MICROCHECKER detected possible null alleles in loci Ase18 and Ase25, thus we excluded them from the same analyses as listed for the reed warbler. For the remaining analyses, we used all eight loci. We found no evidence of large allele dropouts or scoring errors in the data set. The total sample was not in Hardy–Weinberg equilibrium ($\chi^2_{32} = \infty$, $p < 0.001$), nor was Pego-Oliva ($\chi^2_{12} = \infty$, $p < 0.001$) or Mallorca ($\chi^2_{12} = 47.18$, $p < 0.001$). We found no linkage disequilibrium between loci. Expected heterozygosity and allelic richness were higher and inbreeding coefficient lower in the mainland sites of Pego-Oliva and Villafranca than in Mallorca (Table 1b).

The highest support for number of genetic clusters obtained with STRUCTURE was for $K = 3$ (ln likelihood $= -919$). However, at $K = 2$ (ln likelihood $= -935$) we observed a clear peak of ΔK, indicating the strongest structuring into two clusters. In the STRUCTURE bar plot (Fig. 2b, $K = 3$), Mallorca is clearly distinct from the mainland, whereas most of the mainland individuals showed about equal proportions to belong into two clusters. Thus, we identified two clusters corresponding to Mallorca and the Spanish mainland. Pairwise F_{ST} values were significant between all sampling sites, as well as between the mainland and Mallorca (Table 2b). We did not find any indications of isolation by distance (correlation between the Loiselle kinship coefficients and logarithms of distances $r = 0.000$, $p = 0.825$). The assignment analysis assigned 42 out of 54 (78%) individuals to their sampling sites, and when sampling sites were divided to mainland and Mallorca, 48 out of 54 birds (89%) were assigned to their

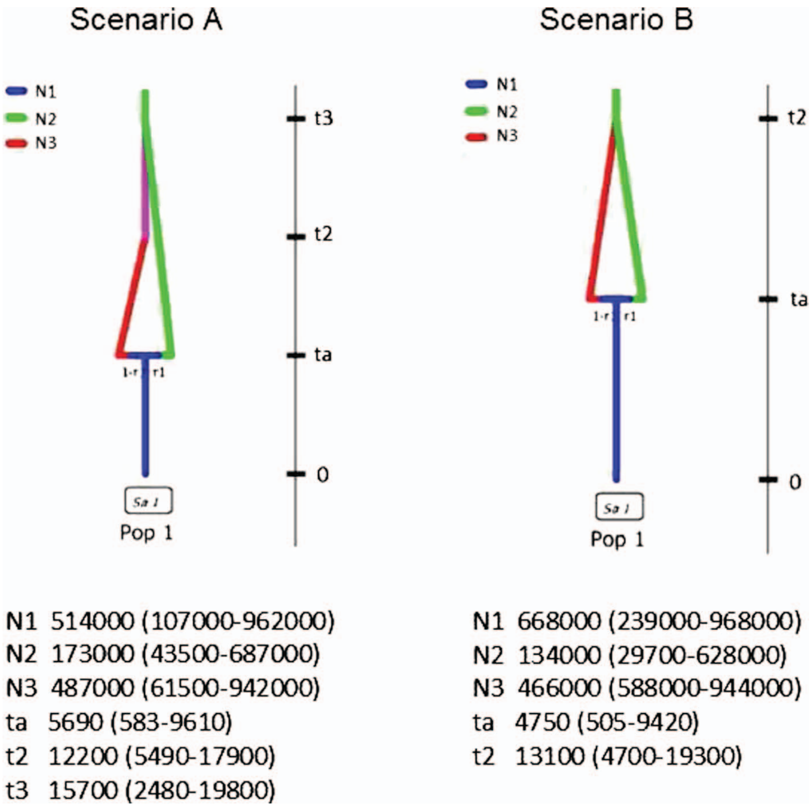

Figure 5. Demographic history of reed warbler according to the two best DIY ABC scenarios obtained from mitochondrial DNA data. The parameters estimates are provided as median (95% CI) and include the current (N1) and the ancient (N2, N3) effective population size, and timing (in terms of generations) of the admixture event (ta), population size variation (t2 scenario A) and population divergence (t3 scenario A, t2 scenario B).

cluster (Table 3b). Two breeding individuals (one sampled in Mallorca and one in Villafranca) were classified as first generation migrants and assigned to Pego-Oliva. The first was a female with regressing brood patch and the second a male with evident cloacal protuberance.

We found signs of recent population size reduction in Mallorca (BOTTLENECK; Wilcoxon test, heterozygosity excess, $p = 0.023$; shifted allele frequency class mode) and less clearly for the Spanish mainland as a whole (Wilcoxon test, heterozygosity excess, $p = 0.039$; normal L-shape of allele frequency distribution). No evidence of past bottlenecks was found, when mainland samples were analysed separately. G–W indices were always > 0.8.

Out of the five simulated scenarios for demographic history tested with the program DIY ABC, the best was the simplest one (Fig. 6), with divergence at time t_1 and no effective population size (N_e) change after divergence. Posterior probabilities of this scenario were 0.970 according to direct estimation and 0.991 according to logistic approach. The model checking based on seven summary statistics and the PCA analysis supported the reliability of the scenario, with the exception of two summary statistics (mean M index, Garza and Williamson 2001; $(\delta\mu)^2$ distance, Goldstein et al. 1995) where posterior distributions deviated ($0.01 > p > 0.001$) from the observed data. The estimate of divergence time was 230 generations (95% CI 41–449), corresponding to 690 yr using the same generation time of 3 yr as with reed warblers. N_e estimate for the Spanish mainland was 4460 (95% CI 1240–6360), and 1500 for Mallorca (95% CI 290–2740). The ancient population size (before divergence) was estimated to be 5890 (95% CI 1430–7820).

Mitochondrial DNA

The 43 sequenced moustached warblers belonged to seven different haplotypes. Similarly to the reed warbler, the TCS cladogram (Fig. 3b) showed a star-like structure, suggesting a past population expansion. The dominant haplotype was found from 35 individuals (81% of the total), while the other six haplotypes were represented by one or two birds. Compared to the reed warbler, haplotype and nucleotide diversities (Table 4b) are markedly lower.

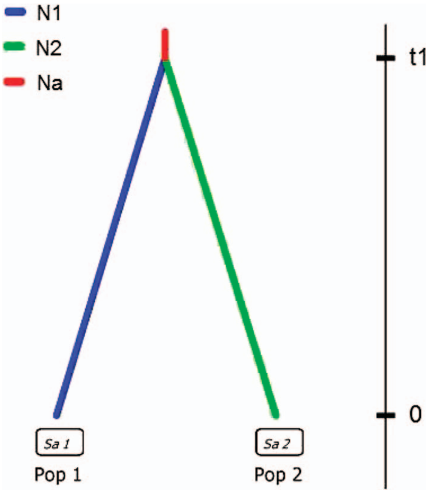

Figure 6. Demographic history of moustached warbler: best DIY ABC scenario obtained from microsatellite data. Divergence time (t1) of the two populations (Pop 1 = Spanish mainland and Pop 2 = Mallorca) is estimated to be 230 generations.

The φ_{ST}-value estimated between mainland Spain and Mallorca was 0.0862 ($p = 0.022$). The pairwise φ_{ST} value was significant also between Pego-Oliva and Mallorca (Table 5b), but low and non-significant between Pego-Oliva and Villafranca. The results of neutrality tests suggested a past expansion for the whole sample ($D = -1.954$, $p < 0.05$; $Fs = -5.308$, $p = 0.004$). The mismatch distribution did not reveal significant deviations from the expansion hypothesis.

Discussion

Despite the large distances between our sampling sites, we detected gene flow for both reed and moustached warblers, although partly restricted or even limited. Our results suggest that both species are able to avoid the risk of isolation derived from breeding in fragmented habitats. However, there were differences between the species; genetic differentiation in reed warblers is lower and diversity higher than in moustached warblers and their population histories differ.

Reed warbler

Our results indicate low genetic differentiation and high gene flow between the sampling sites, suggesting high dispersal capability for the reed warblers. Low differentiation of the reed warbler populations has been observed also previously in Europe (Kralj et al. 2010, Procházka et al. 2011). However, the weak but significant differentiation among some of the sampling sites in microsatellite data indicates that gene flow can be partly restricted. This can be due to the relatively large distances between sampling sites (from ca 290 to 960 km) or breeding site fidelity reported for this species, especially for adult birds (Vadász et al. 2008). Yet, lack of isolation by distance did not clearly support this. It is also possible that the result is affected by small sample sizes.

Given the lack of a clear structuring between Spain (subspecies *scirpaceus*) and Morocco in both markers, our results do not support the hypotheses that the Moroccan population should form a separate taxon (see Introduction; Amezian et al. 2010, Jiguet et al. 2010). In fact, Iberian and Moroccan birds even shared the same haplotypes. Besides the geographical proximity of the two areas, Morocco is crossed by the migration routes of the European SW-migrating populations, which includes the Iberian reed warblers (Procházka et al. 2008), which is likely to facilitate the gene flow between these populations.

We found signs of past population growth and mixing of previously isolated populations. The dating of this population admixture calculated in DIY ABC (Fig. 5) is compatible with a postglacial expansion from a refugium area, as the climate became warmer, causing the expansion of the suitable habitat also for many other species (Murray Gates 1993, Wright et al. 1993, Hewitt 2000, Dubey et al. 2006). Arbabi et al. (2014) identified three mitochondrial lineages corresponding to three subspecies (*scirpaceus*, *fuscus* and *avicenniae*) of reed warblers and suggested these had remained isolated in three different glacial refugia during the Middle

Pleistocene glaciations. Procházka et al. (2011) suggested that it is possible that the Iberian Peninsula was one of the refugium areas for reed warblers, as has been documented for many other species (Hewitt 2004). Evidence of past population growth in reed warblers was detected also by Arbabi et al. (2014), but their samples were mainly from Germany.

The haplotype and nucleotide diversities we obtained were higher ($n = 50$; $h = 0.745$; $\pi = 0.006$) than estimated for Germany ($n = 347$; $h = 0.544$; $\pi = 0.002$) or for subspecies *scirpaceus* ($n = 380$; $h = 0.558$; $\pi = 0.002$) in Arbabi et al. (2014). The higher genetic diversity could be linked to an older population, i.e. more ancient refugium area (Comes and Kadereit 1998, Taberlet et al. 1998) than suggested by Arbabi et al. (2014). However, in this case the high diversity obviously results from admixture between the two differentiated lineages. The population divergence preceding the admixture (Fig. 5) can be dated to about 47 000–40 000 yr ago (given a generation time of three years) and thus coincides with the MS3 stage of the last Glacial.

Moustached warbler

Microsatellite and mtDNA data indicate that gene flow is limited between the Spanish mainland and Mallorca Island and possibly restricted also among the two mainland sites. The assignment test suggest that occasional dispersal occurs from the mainland to Mallorca, but not much vice versa. However, it is possible that the classification of a breeding bird as a first generation migrant is not due to a real dispersal event, but to false detection due to small sample size or due to the sampling sites not being in H–W equilibrium (Excoffier and Heckel 2006).

Approximately the same distance (ca 290 km) separates Pego-Oliva from Villafranca and Mallorca, but only the Mallorcan sample was clearly distinct. This suggests that gene flow between the Spanish mainland and Mallorca is likely to be limited not only by the distance, but also by the sea (ca 200 km wide) between the coast and the island. Thus, our results suggest that the sea forms an effective dispersal barrier and limits gene flow in moustached warblers, at least between our study sites. For a warbler, crossing the sea means a lack of resting and foraging opportunities until reaching land, which is more risky and difficult than dispersal over land. The low differentiation among the mainland sites indicates gene flow between the important breeding populations of the Mediterranean coast and the small and scattered inland populations breeding in Castilla-La Mancha (estimated to be only 10 pairs by Castany and López 2006), represented by Pego-Oliva and Villafranca samples in our study, respectively. The breeding bird sampled in Villafranca and classified as first generation migrant from Pego-Oliva can be a further sign of dispersal. In any case, the gene flow is not high, possibly because the two mainland sites are divided by a wide area without any other known breeding populations (Castany and López 2006) and without suitable habitat. As in reed warblers, breeding site fidelity in moustached warblers (Vadász et al. 2008) could also reduce dispersal.

mtDNA showed evidence of past population growth, likely a sign of postglacial expansion. As discussed above about reed warblers, moustached warbler populations were probably confined to glacial refugia and extended their range across Europe when the climate became warmer. Taking into account the population structuring, lower genetic variation and bottleneck and coalescence analysis, we suggest that Mallorca has been recently colonized or re-colonized by individuals originating from the mainland. After the settlement of the founding population, there is still some limited gene flow from the mainland.

Differences in population structure and demographic history between the two species – possible explanations

The lower genetic differentiation found in reed warblers indicates a higher dispersal rate than in moustached warblers. Long-distance migrants, like the reed warbler, are suggested to have higher dispersal ability than sedentary or short-distance migrants, such as the moustached warbler (Gill et al. 1993, Lovette et al. 1998, Paradis et al. 1998, Arguedas and Parker 2000). Differences in wing morphology has been suggested to be connected with migratory behaviour, as migratory species tend to have more pointed wings than sedentary species (Mönkkönen 1995). This is also seen in our study species; the reed warblers have more pointed wings than the moustached warblers (Kennerley and Pearson 2010) allowing greater flight efficiency (Norberg 1989). In addition, our results suggest that differences in dispersal ability between reed and moustached warblers can result to different capability in crossing natural barriers, in this case dispersal over sea. The Mediterranean Sea is along the migration routes of the reed warblers and thus, it is obligatory for this species to be able to cross these kinds of barriers. On the contrary, migration of Spanish and French populations of moustached warblers mostly follow the Mediterranean coast (Castany 2003) and do not require crossing the sea, thus overcoming such a barrier is probably less common for these birds.

Higher differentiation in moustached warblers could be also due to the smaller population size and the more discontinuous breeding range than in reed warblers. In fact, breeding areas of moustached warblers are more isolated from each other, making the exchange of individuals more difficult. In addition, Vadász et al. (2008) reported higher breeding site fidelity in moustached than in reed warblers. They hypothesized that the more specialized habitat preferences of moustached warblers limit the opportunities to find new suitable areas, resulting in reduced dispersal rates (Vadász et al. 2008). Similar studies of the west Mediterranean moustached and reed warbler populations would be needed to assess the breeding site fidelity.

Both species showed signs of postglacial expansions, consistently with the findings of other studies of European wetland passerines (Hansson et al. 2008, Neto et al. 2012, Arbabi et al. 2014). Haplotype diversity was higher for reed warblers, possibly because of a more important contribution of dispersal from different populations, past admixture of differentiated lineages and the higher population size (Fig. 3, 5 and 6). The haplotype network structures of reed and moustached warblers (Fig. 3) resemble each other, but whereas the reed warbler tree shows mixing of distinct lineages within populations, the other lineage of moustached warblers was found only from the Russian individual used as an outgroup. This indicates that the postglacial expansion and colonisation of the present ranges of these species occurred in dissimilar ways.

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References

Amezian, M., Cortes, J., Thompson, I., Bensusan, K., Perez, C., Louah, A., El Agbani, M. A. and Qninba, A. 2010. Complete moult of an undescribed resident taxon of the reed warbler *Acrocephalus scirpaceus/baeticatus* complex in the Smir marshes, northern Morocco. – Ardea 98: 225–234.

Arbabi, T., Gonzalez, J., Witt, H.-H., Klein, R. and Wink, M. 2014. Mitochondrial phylogeography of the Eurasian reed warbler *Acrocephalus scirpaceus* and the first genetic record of *A. s. fuscus* in central Europe. – Ibis doi: 10.111/1ibi.12174

Arguedas, N. and Parker, P. G. 2000. Seasonal migration and genetic population structure in house wrens. – Condor 102: 517–528.

Bensch, S. and Hasselquist, D. 1999. Phylogenetic population structure of great reed warblers: an analysis of mtDNA control region sequences. – Biol. J. Linn. Soc. 66: 171–185.

Bensch, S., Price, T. and Kohn, J. 1997. Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. – Mol. Ecol. 6: 91–92.

BirdLife International 2012. *Acrocephalus scirpaceus*. – IUCN 2013, IUCN Red List of threatened species, ver. 2013.2, <www.iucnredlist.org> accessed 6 June 2014.

Bohonak, A. J. 1999. Dispersal, gene flow, and population structure. – Q. Rev. Biol. 74: 21–45.

Carrascal, L. M. and Palomino, D. 2008. Las aves comunes reproductoras en España. Población en 2004–2006. – SEO/BirdLife.

Castany, J. 2003. El carricerín real (*Acrocephalus melanopogon*) en el P. N. del Prat de Cabanes-Torreblanca. – PhD thesis, Univ. of Valencia.

Castany, J. and López, G. 2006. El carricerín real en España. I Censo Nacional (2005). – SEO/BirdLife.

Clement, M., Posada, D. and Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. – Mol. Ecol. 9: 1657–1660.

Comes, H. P. and Kadereit, J. W. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. – Trends Plant Sci. 3: 432–438.

Cornuet, J. M. and Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. – Genetics 144: 2001–2014.

Cornuet, J. M., Santos, F., Beaumont, M. A., Robert, C. P., Marin, J. M., Balding, D. J., Guillemaud, T. and Estoup, A. 2008. Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. – Bioinformatics 24: 2713–2719.

Cramp, J. S. 1992. The birds of the Western Palearctic. Vol. 6. – Oxford Univ. Press.

Dawson, D. A., Hanotte, O., Greig, C., Stewart, I. R. K. and Burke, T. 2000. Polymorphic microsatellites in the blue tit *Parus caeruleus* and their cross-species utility in 20 songbird families. – Mol. Ecol. 9: 1941–1944.

Desrochers, A., Hannon, S. J., Bélisle, M. and St Clair, C. C. 1999. Movement of songbirds in fragmented forests: can we scale-up from behaviour to explain occupancy patterns in the landscape? – In: Adams, N. J. and Slotow, R. H. (eds), Proceedings of the 22nd International Ornithological Congress Johannesbourg. BirdLife South Africa, pp. 2447–2464.

Dubey, S., Zaitsev, M., Cosson, J., Abdukadier, A. and Vogel, P. 2006. Pliocene and Pleistocene diversification and multiple refugia in a Eurasian shrew (*Crocidura suaveolens* group). – Mol. Phylogenet. Evol. 38: 635–647.

Ellegren, H. 1992. Polymerase-chain-reaction (PCR) analysis of microsatellites – a new approach to studies of genetic relationships in birds. – Auk 109: 886–895.

Estoup, A., Jarne, P. and Cornuet, J. M. 2002. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. – Mol. Ecol. 11: 1591–1604.

Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. – Mol. Ecol. 14: 2611–2620.

Excoffier, L. and Heckel, G. 2006. Computer programs for population genetics data analysis: a survival guide. – Nat. Rev. Genet. 7: 745–758.

Excoffier, L. and Lischer, H. 2010. Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. – Mol. Ecol. Resour. 10: 564–567.

Falush, D., Stephens, M. and Pritchard, J. K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. – Genetics 164: 1567–1587.

Finlayson, M., Hollis, T. and Davis, T. 1992. Managing Mediterranean wetlands and their birds. – The International Waterfowl and Wetlands Research Bureau Spec. Publ. no. 20, The International Waterfowl and Wetlands Research Bureau.

Frankham, R., Ballou, J. D. and Briscoe, D. A. 2010. Introduction to conservation genetics, 2nd ed. – Cambridge Univ. Press.

Frentiu, F. D., Lange, C. L., Burke, T. and Owens, I. P. F. 2003. Isolation of microsatellite loci in the capricorn silvereye, *Zosterops lateralis chlorocephalus* (Aves: Zosteropidae). – Mol. Ecol. Notes 3: 462–464.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. – Genetics 147: 915–925.

Galbusera, P., Githiru, M., Lens, L. and Matthysen, E. 2004. Genetic equilibrium despite habitat fragmentation in an Afrotropical bird. – Mol. Ecol. 13: 1409–1421.

Garza, J. C. and Williamson, E. G. 2001. Detection of reduction in population size using data from microsatellite loci. – Mol. Ecol. 10: 305–318.

Gibbs, H. L., Tabak, L. M. and Hobson, K. 1999. Characterization of microsatellite DNA loci for a neotropical migrant songbird, the Swainson's thrush (*Catharus ustulatus*). – Mol. Ecol. 8: 1551–1552.

Gill, F. B., Mostrom, A. M. and Mack, A. L. 1993. Speciation in North American chickadees: I. Patterns of mtDNA genetic divergence. – Evolution 47: 195–212.

Goldstein, D. B., Linares, A. R., Cavalli-Sforza, L. L. and Feldman, M. W. 1995. An evaluation of genetic distances for use with microsatellite loci. – Genetics 139: 463–471.

Goudet, J. 1995. FSTAT: a computer program to calculate F-statistics. – J. Hered. 86: 485–486.

Griffith, S. C., Stewart, I. R. K., Dawson, D. A., Owens, I. P. F. and Burke, T. 1999. Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an 'island effect'? – Biol. J. Linn. Soc. 68: 303–316.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucl. Acids Symp. Ser. 41: 95–98.

Hansson, B., Bensch, S., Hasselquist, D., Lillandt, B. G., Wennerberg, L. and von Schantz, T. 2000. Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNS fingerprinting. – Mol. Ecol. 9: 1529–1538.

Hansson, B., Bensch, S., Hasselquist, D. and Nielsen, B. 2002. Restricted dispersal in a long-distance migrant bird with patchy distribution, the great reed warbler. – Oecologia 130: 536–542.

Hansson, B., Hasselquist, D., Tarka, M., Zehtindjiev, P. and Bensch, S. 2008. Postglacial colonisation patterns and the role of isolation and expansion in driving diversification in a passerine bird. – PLoS One 3: e2794.

Hardy, O. J. and Vekemans, X. 2002. SPAGeDi: a versatile computer program to analyze spatial genetic structure at individual or population level. – Mol. Ecol. Notes 2: 618–620.

Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S. and Francis, C. M. 2004. Identification of birds through DNA barcodes. – PLoS Biol. 2: 1657–1688.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. – Nature 405: 907–913.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. – Proc. R. Soc. B 359: 183–195.

Jiguet, F., Rguibi-Idrissi, H. and Provost, P. 2010. Undescribed reed warbler breeding in Morocco. – Dutch Birding 32: 29–36.

Kennerley, P. and Pearson, D. 2010. Reed and bush warblers. – Christopher Helm Publishers.

Kerr, K. C. R., Stoeckle, M. Y., Dove, C. J., Weigt, L. A., Francis, C. M. and Hebert, P. D. N. 2007. Comprehensive DNA barcode coverage of North American birds. – Mol. Ecol. Notes 7: 535–543.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. – J. Mol. Evol. 16: 111–120.

Koenig, W. D., Van Vuren, D. and Hooge, P. N. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. – Trends Ecol. Evol. 11: 514–517.

Kralj, J., Procházka, P., Fainová, D., Patzenhauerová, H. and Tutiš, V. 2010. Intraspecific variation in the wing shape and genetic differentiation of reed warblers *Acrocephalus scirpaceus* in Croatia. – Acta Ornithol. 45: 51–58.

Kvist, L., Ponnikas, S., Belda, E. J., Encabo, I., Martínez, E., Onrubia, A., Hernández, J. M., Vera, P., Neto, J. M. and Monrós, J. S. 2011. Endangered subspecies of the reed bunting (*Emberiza schoeniclus witherby* and *E. s. lusitanica*) in Iberian Peninsula have different genetic structures. – J. Ornithol. 152: 681–693.

Laurence, W. F. 2010. Habitat destruction: death by a thousand cuts. – In: Sohdi, N. S. and Ehrlich, P. R. (ed.), Conservation biology for all. Oxford Univ. Press, pp. 73–87.

Librado, P. and Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. – Bioinformatics 25: 1451–1452.

Loiselle, B. A., Sork, V. L., Nason, J. and Graham, C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). – Am. J. Bot. 82: 1420–1425.

Lovette, I. J., Bermingham, E., Seutin, G. and Ricklefs, R. E. 1998. Evolutionary differentiation in three endemic West Indian warblers. – Auk 115: 890–903.

Martínez, J. G., Soler, J. J., Soler, M., Møller, A. P. and Burke, T. 1999. Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). – Evolution 53: 269–278.

Matthysen, E., Lens, L., Van Dongen, S., Verheyen, G. R., Wauters, L. A., Adriaensen, F. and Dhondt, A. A. 1995. Diverse effects of forest fragmentation on a number of animal species. – Belg. J. Zool. 125: 175–183.

Mönkkönen, M. 1995. Do migrant birds have more pointed wings? A comparative study. – Evol. Ecol. 9: 520–528.

Murray Gates, D. 1993. Climate change and its biological consequences. – Sinauer Associates.

Neto, J. M., Arroyo, J. L., Bargain, B., Monrós, J. S., Mátrai, N., Procházka, P. and Zehtindjiev, P. 2012. Phylogeography of a habitat specialist with high dispersal capability: the Savi's warbler *Locustella luscinioides*. – PLoS One 7: e38497.

Neumann, K. and Wetton, J. H. 1996. Highly polymorphic microsatellites in the house sparrow *Passer domesticus*. – Mol. Ecol. 5: 307–309.

Newton, I. 1998. Population limitation in birds. – Academic Press.

Norberg, U. M. 1989. Vertebrate flight, mechanics, physiology, morphology, ecology and evolution. – Springer.

Paetkau, D., Slade, R., Burden, M. and Estoup, A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. – Mol. Ecol. 13: 55–65.

Paracuellos, M. and Tellería, J. L. 2004. Factors affecting the distribution of a waterbird community: the role of habitat configuration and bird abundance. – Waterbirds 27: 446–453.

Paradis, E., Baillie, S. R., Sutherland, W. J. and Gregory, R. D. 1998. Patterns of natal and breeding dispersal in birds. – J. Anim. Ecol. 67: 518–536.

Peiró, I. G. 2003. Intraspecific variation in the wing shape of the long-distance migrant reed warbler *Acrocephalus scirpaceus*: effects of age and distance of migration. – Ardeola 50: 31–37.

Petren, K. 1998. Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches. – Mol. Ecol. 7: 1782–1784.

Piry, S., Luikart, G. and Cornuet, J. M. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. – J. Hered. 95: 536–539.

Piry, S., Alapetite, A., Cornuet, J. M., Paetaku, D., Baudouin, L. and Estoup, A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. – J. Hered. 95: 536–539.

Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. – Genetics 155: 945–959.

Procházka, P., Hobson, K. A., Karcza, Z. and Kralj, J. 2008. Birds of a feather winter together: migratory connectivity in the reed warbler *Acrocephalus scirpaceus*. – J. Ornithol. 149: 141–150.

Procházka, P., Stokke B. G., Jensen, H., Fainová, D., Bellinvia, D., Fossøy, V. J. R., Bryja, J. and Soler, M. 2011. Low genetic differentiation among reed warbler *Acrocephalus scirpaceus* populations across Europe. – J. Avian Biol. 42: 103–113.

Rannala, B. and Mountain, J. L. 1997. Detecting immigration by using multilocus genotypes. – Proc. Natl Acad. Sci. USA 94: 9197–9201.

Raymond, M. and Rousset, F. 1995. GENEPOP (version 1.2): population genetic software for exact tests and ecumenism. – J. Hered. 86: 248–249.

Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. and Burke, T. 2000. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphicin Sylvidae species and their cross-species amplification in other passerine birds. – Mol. Ecol. 9: 2225–2230.

Rogers, A. R. and Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. – Mol. Biol. Evol. 9: 552–569.

Rousset, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. – Mol. Ecol. Resour. 8: 103–106.

Silva, J. P., Phillips, L., Jones, W., Eldridge, J. and O'Hara, E. 2007. Life and Europe's wetlands, restoring a vital ecosystem. – Office for Official Publications of the European Communities.

Spurgin, L. G., Wright, D. J., van der Velde, M., Collar, N. J., Komdeur, J., Burke, T. and Richardson, D. S. 2014. Museum DNA reveals the demographic history of the endangered Seychelles warbler. – Evol. Appl. 7: 1134–1143.

Taberlet, P., Fumagalli, L., Wust-Saucy, A. G. and Cosson J. F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. – Mol. Ecol. 7: 453–464.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. – Genetics 123: 585–595.

Vadász, C., Német, Á., Karcza, Z., Loránt, M., Biró, C. and Csörgő, T. 2008. Study on breeding site fidelity of *Acrocephalus* warblers in central Hungary. – Acta Zool. Acad. Sci. Hung. 54 (Suppl. 1): 167–175.

van Oosterhout, C. V., Hutchinson, W. F. and Wills, D. P. M. 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. – Mol. Ecol. Notes 4: 535–538.

Van Vessem, J., Hecker, N. and Tucker, G. M. 1997. Inland wetlands. – In: Tucker, G. M. and Evans, M. I. (eds), Habitats for birds in Europe: a conservation strategy for the wider environment. BirdLife Conservation Series 6. BirdLife International, pp. 125–158.

Wright, H. E., Kutzbach, J. E., Webb, T., Ruddiman, W. F., Street-Perrott, F. A. and Bairlein, P. J. 1993. Global climates since the Last Glacial Maximum. – Univ. of Minnesota Press.

Supplementary material (Appendix JAV-00589 at <www.avianbiology.org/readers/appendix>). Appendix 1.

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Supplementary material

Appendix 1

PCR procedures

Microsatellites

For both reed and moustached warblers, we performed the Polymerase Chain Reaction (PCR) in 10-µl volumes using 2 µl of DNA template, 1 µl of 10x PCR-buffer, 0.9 µl of dNTPs (2 mM), 0.8 of primers (10 µM) (or 0.4 of 20 µM primer), 0.4 of $MgCl_2$ (2 mM) and 0.1 µl of DNA-polymerase (Biotools). The PCR procedure consisted of an initial denaturation for 5 min (94°C), 35 cycles of denaturation for 45 s (94°C), annealing for 45 s (from 48° to 63°C) and synthesis for 1 min (72°C), and a final synthesis for 7 min (72°C). Annealing temperatures for the primers were 50°C for Gf05 and Pdo5, 52°C for Ppi2, 54°C for Cuµ28 and ZL54, a touchdown from 60° to 50°C for Pocc2, from 63° to 53°C for Aar5, FhU2, Pca3 and Pdoµ1, from 54° to 48°C for Aar4 and Ase34, and from 60° to 54°C for Ase18, Ase25, Ase37, Ase48 and Ase58.

Mitochondrial DNA

For reed warblers, we performed the PCR in 10-µl volumes using 2 µl of DNA template, 1 µl of 10x PCR-buffer, 0.5 µl of MgCl2 (2 mM), 1 µl of dNTPs (2 mM), 0.4 µl primer CO1F (20 µM), 0.4 µl of primer CO1R (20 µM) and 0.06 µl of DNA-polymerase (Biotools). We used the following PCR profile: denaturation for 1 min (95°C), 35 cycles of denaturation for 30 s (94°C), annealing for 45 s (49°C) and synthesis for 45 s (72°C), and a final synthesis for 10 min (72°C).

For moustached warblers, we carried out the PCR in 10-µl volumes using 5 µl of DNA template, 1 µl of 10x PCR-buffer, 0.4 µl of MgCl2 (2 mM), 1 µl of dNTPs (2mM), 0.4 µl primer CO1F (20 µM), 0.4 µl of primer CO1R2 (20 µM) and 0.06 µl of DNA-polymerase (Biotools). We used the same PCR profile employed for reed warblers, but with 50°C as annealing temperature.

Chapter 4

Similar dispersal patterns between two closely related birds with contrasting migration strategies

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ORIGINAL ARTICLE

Similar dispersal patterns between two closely related birds with contrasting migration strategies

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Abstract Studying dispersal is crucial to understand metapopulation and sink-source dynamics and invasion processes. The capability to disperse is especially important for species living in fragmented habitats like wetlands. We investigated the distribution of natal and breeding dispersal distances and philopatry in Spanish populations of two closely related reedbed-nesting birds, the Moustached Warbler *Acrocephalus melanopogon* and the Eurasian Reed Warbler *Acrocephalus scirpaceus*. These warblers are morphologically very similar, but differ in migration strategy and, in our study area, in population size. Our aims were to find the best model for dispersal distances and to assess the occurrence of intra- or interspecific differences in dispersal patterns. We used ringing data from the Spanish marking scheme and selected recaptures to avoid including migrating individuals. In both species, most individuals were philopatric but dispersing birds were able to cross large distances (up to more than 100 km), suggesting the capability to compensate for habitat fragmentation. We found the heavy-tailed Cauchy distribution to be the best conceptual description for our data, in all cases but natal dispersal of Moustached Warblers. Among Eurasian Reed Warblers, natal philopatry was lower than breeding philopatry. We found no significant interspecific differences. This does not confirm the hypothesis of higher dispersal ability in long distance migrants (like Eurasian Reed Warblers) than in resident/short distance migrant bird species (like Moustached Warblers). The similarity in dispersal patterns among the two warblers may be explained by their close phylogenetic relatedness, similar constraints imposed on both species by a patchy habitat or similar evolutionary pressures.

Keywords *Acrocephalus melanopogon* · *Acrocephalus scirpaceus* · Dispersal · Long-distance dispersal · Philopatry · Spatial ecology

Introduction

Studying dispersal is crucial in population biology. It is an important element in metapopulation and sink-source dynamics (Begon et al. 2006), in colonization and invasion processes (Hengeveld 1994; Shigesada et al. 1995), and should be taken into account when studying the processes underlying adaptation (Lambrechts et al. 1999). Dispersal is also related to gene flow and, consequently, to the degree of genetic differentiation among populations (Bohonak 1999; Frankham et al. 2010). Thus, information about dispersal is of critical importance for conservation, especially for species living in fragmented habitats (e.g., Van Houtan et al. 2010). Dispersal in birds has been the object of many studies, but the high movement capability of most bird species implies important problems of data collection (Koenig et al. 1996; Paradis et al. 1998 and references therein). Indeed, small-scale study areas are likely to underestimate the frequency of long-distance dispersal events (Koenig et al. 1996; Paradis et al. 1998). In spite of being relatively rare, such events are likely to be important in population dynamics (Nathan et al. 2003) and in determining the genetic structure of populations (Ibrahim et al.

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1996). A ringed bird may be recovered anywhere, thus recapture data from ringing databases are not spatially limited and can provide useful information about dispersal (Paradis et al. 1998, 2002).

In this study, we used data from the Spanish marking scheme to investigate large-scale dispersal patterns of two closely related reedbed-nesting birds, the Eurasian Reed Warbler *Acrocephalus scirpaceus* Hermann 1804 and the Moustached Warbler *Acrocephalus melanopogon* Temminck 1823. These species are similar in size (approximately 10 g) and are both insectivorous, but they differ in migration strategy. Their population sizes in Spain are also highly different. The Eurasian Reed Warbler (hereafter Reed Warbler) is a common breeding bird in Spain (679,000–1320,000 individuals, Carrascal and Palomino 2008) and migrates over long distances, wintering in sub-Saharan Africa (Kennerley and Pearson 2010). The Spanish population of Moustached Warbler is sedentary or migrate over short distances, is distributed across a highly discontinuous range and has been estimated in 484–1777 breeding pairs (Castany and López 2006). Distribution maps of both species in Spain are available at *Atlas Virtual de las Aves Terrestres de España* (Carrascal et al. 2005; http://www.lmcarrascal.eu/atlas/mapasalfab.html). The breeding habitat of both species is highly fragmented, because of the natural patchy distribution of wetlands and the habitat loss due to human activities (Van Vessem et al. 1997; Paracuellos and Tellería 2004; Silva et al. 2007).

Our aims were: (1) to obtain new information about dispersal ability and philopatry in Spanish populations of the two species, the Reed Warbler and the Moustached Warbler; (2) to find the best conceptual description of dispersal distances in these warblers, among a set of simple models (one-two parameters, see Table 1 for details) with clear biological meaning; (3) to assess the occurrence of intraspecific differences in dispersal patterns, between natal dispersal (from the birth place to a breeding site) and breeding dispersal (between breeding sites); (4) to assess the occurrence of interspecific differences in dispersal patterns between the two species: long-distance migrants are suggested to have higher dispersal ability than sedentary/short-distance migrant species (Paradis et al. 1998). Genetic data suggest that this pattern also occurs among our study species, given the higher differentiation among Spanish populations of Moustached Warbler than among Reed Warblers breeding in Spain and Morocco (Ceresa et al. 2015). In addition, Reed Warblers have more pointed wings than Moustached Warblers (Kennerley and Pearson 2010), indicating higher flight efficiency (Norberg 1990) and, consequently, a possibly higher dispersal capability. Concerning our model set, we fitted distance data to the normal, the exponential and the Cauchy distributions. The normal distribution describes a low diffusion through space, determined by various external stochastic factors ('random walk'; Van Houtan et al. 2007). Dispersal distances will follow an exponential distribution if moving individuals have a constant probability of settling (Paradis et al. 2002). Finally, the Cauchy distribution is heavy-tailed, and as such it predicts more frequent long-distance dispersal events than the exponential distribution (Paradis et al. 2002).

Methods

Data selection

The starting database was made available by 'Banco de datos de anillamiento del remite ICONA—Ministerio de Medio Ambiente' (2015). It included all recoveries of individuals ringed in Spain (including those birds recovered outside the country) from year 1962 to 2013 and provided recovery distances to the nearest kilometre. The ringing effort on the two species increased throughout the years, up to reaching the maximum number of marked individuals in the decade 2001–2010 (6425 Moustached Warblers and 229,671 Reed Warblers; SEO/BirdLife 2016a, b). Consequently, also the majority of the collected recovery data (>80 %) are referred to birds ringed during that decade and the following years. Ringing and recoveries occurred at overall 1127 different localities, among these the Moustached Warbler was recorded at 103 sites and the Reed Warbler at 1099 sites.

In this study, we only considered birds ringed during the breeding season and recovered during the breeding season of following years. We defined two age classes: juveniles for individuals ringed in their year of birth and adults for birds ringed later. Natal dispersal was estimated by using birds ringed as juveniles, while to estimate breeding dispersal we used birds ringed as adults (Paradis et al. 1998). We excluded those birds whose age was unknown when ringed. Periods adopted as breeding season were 1 June–31

Table 1 Distributions adopted for modelling dispersal distances (*d*)

Distribution	Probability density function	Parameters (name)
Normal	$\frac{1}{\sigma\sqrt{2\pi}}e^{-(d-\mu)^2/2\sigma^2}$	μ (mean), σ (standard deviation)
Exponential	$\lambda e^{-\lambda d}$	λ (rate)
Cauchy	$\frac{1}{\pi\gamma}\left[\frac{\gamma^2}{(d-x)^2+\gamma^2}\right]$	x (location), γ (scale)

July for the Reed Warbler (Cantos and Tellería 1994) and 1 April–31 May for the Moustached Warbler (Castany 2003). Juvenile Reed Warblers ringed in May (early fledged individuals) were also included. We checked the geographical coordinates provided in the database to ensure they corresponded to the ringing/recovery localities, when they did not we corrected them. Given the intrinsic characteristics of our dataset, we could not investigate fine-scale movements of birds within each breeding area, thus all dispersal events described in this work consist in displacements from a natal/breeding site towards a different patch of suitable habitat. To obtain reliable information about dispersal distances, it was very important to avoid including individuals ringed/recovered when migrating. The periods we adopted as breeding season excluded the main migration periods of the study species (Cantos and Tellería 1994; Castany 2003; Peirò 2003; Kennerley and Pearson 2010), but even so we risked including some late/early migrating individuals. Examining recoveries of Reed Warblers, we observed that the direction of most of the largest recorded distances approached or corresponded to the North-East–South-West axis, followed by most individuals migrating through the Iberian Peninsula (e.g., Procházka et al. 2011). We then discarded the largest dispersal distances when orientated along the NE–SW axis (Paradis et al. 1998). As a result of this selection, all dispersal distances larger than 290 km were removed and recoveries were not preferentially oriented along this axis (ḿigratory axis' vs. all other directions: $\chi^2_1 = 1.041$, $P = 0.308$). Including all the data did not anyway affect the results of our study (i.e., we obtained very similar outputs from data analysis). For Moustached Warblers we found a low number of dispersing individuals, thus we could not assess if distances were preferentially orientated in some direction. We then used all available recoveries for this species. A further possible risk was to include birds that were not actually breeding when recaptured, e.g., in several passerine species some individuals do not breed during their first potential breeding season (Cooper et al. 2009 and references therein). However, such individuals are likely to be waiting for an opportunity to obtain a territory/mate (Newton 1992) or looking for extra-pair copulations (see, e.g., Blomqvist et al. (2005) for the Moustached Warbler). Thus, even when occurring in our dataset, such individuals were probably recovered close to their potential breeding sites (Paradis et al. 1998).

Statistical analysis

Within the set of selected recoveries, we first calculated the proportion of philopatric and dispersing individuals among juveniles and adults of the two species and carried out inter- and intraspecific comparisons by means of Chi square (χ^2) test of independence (Winkler et al. 2004). To compare the distribution of dispersal distances among species and age classes we used the two-samples Kolmogorov–Smirnov test. Interspecific comparisons were carried out by considering natal and breeding dispersal both jointly (i.e., using all data per species) and separately. In order to find the best dispersal model among our set (see "Introduction") we fitted dispersal distances to the normal, the exponential and the Cauchy distributions using package fitdistrplus 1.0–4 (Delignette-Muller and Dutang 2015) in R 3.1.1 (R Core Team 2014). For the Cauchy distribution, we always fixed the location parameter at $x = 0.1$. We ranked models using the Akaike Information Criterion (AIC), and considered the model with lower AIC as the best one (Burnham and Anderson 2002).

Ideally, spatial variation in sampling effort should be taken into account when studying the distribution of dispersal distances (e.g., Van Houtan et al. 2007), because it is likely to determine a spatial variation in recovery rates, but we lacked the necessary data. As suggested by Paradis et al. (1998), the bias resulting form spatial variation in recovery rates may be reduced if the number of potential recovery sites is large (like in this study), and interspecific comparisons are unlikely to be affected when species are studied over the same area, because spatial variation in recovery rates would be similar.

Results

Both natal and breeding philopatry were high in both species: the proportion of philopatric individuals was 98 and 92 % respectively in juvenile and adults Moustached Warblers, 91 and 94 % in juvenile and adult Reed Warblers. Among Reed Warblers, natal philopatry was lower than breeding philopatry ($\chi^2_1 = 6.273$, $P = 0.012$), while we found no significant difference among Moustached Warblers ($\chi^2_1 = 1.066$, $P = 0.302$) or among species (all data: $\chi^2_1 = 0.128$, $P = 0.721$; juveniles: $\chi^2_1 = 2.285$, $P = 0.131$; adults: $\chi^2_1 = 0.233$, $P = 0.629$). Some of the relatively rare dispersing individuals were recovered at long distance (Fig. 1); birds dispersing further than 100 km were found in all categories excepted the juvenile Moustached Warblers (the category with lower sample size, $n = 50$). According to the Kolmogorov–Smirnov test we found no significant difference in distribution of dispersal distances among the two species (all data: $D = 0.026$, $P = 1$; juveniles: $D = 0.075$, $P = 0.963$; adults: $D = 0.030$, $P = 1$) and among age classes (Reed Warbler: $D = 0.041$, $P = 0.590$; Moustached Warbler: $D = 0.06$, $P = 1$). According to the AIC values, the Cauchy distribution provided the best model for dispersal distances in all cases, excepted the natal dispersal of Moustached Warblers

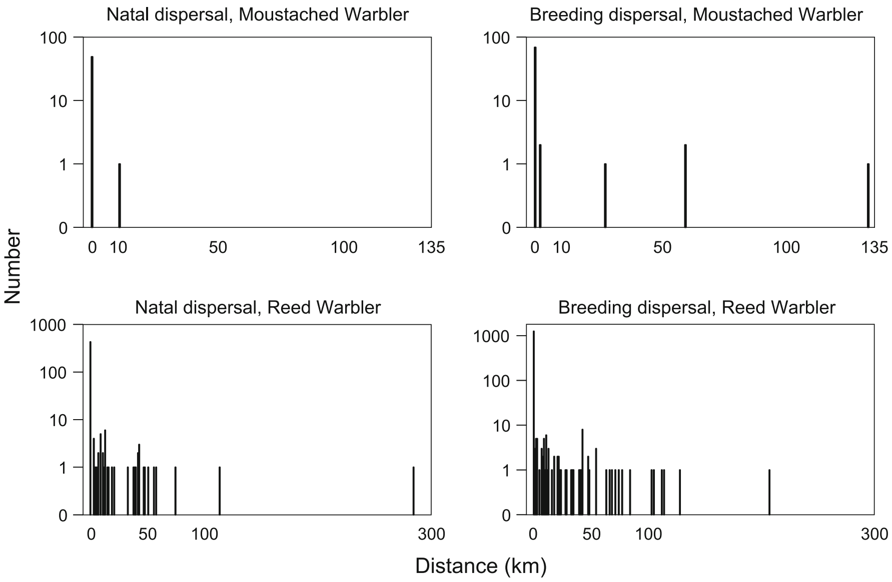

Fig. 1 Natal and breeding dispersal distances in Spanish populations of Moustached and Reed Warbler, obtained from ringing data. The *x*- and *y*-axis scales are different among the panels

Table 2 AIC values for three dispersal models (the best model for each data category is highlighted in bold), sample size (*n*) and the estimated Cauchy scale parameter (γ) with the 95 % confidence intervals (CI)

Species	Data category	*n*	AIC values			γ (95 % CI)
			Cauchy	Exponential	Normal	
Reed	All data	1808	**693**	6509	14,466	0.108 (0.102, 0.113)
Warbler	Natal dispersal	476	**416**	1961	4017	0.111 (0.101, 0.122)
	Breeding dispersal	1332	**278**	4500	10,372	0.106 (0.101, 0.112)
Moustached	All data	125	**20**	468	1022	0.106 (0.087, 0.125)
Warbler	Natal dispersal	50	–27	**−53**	185	0.102 (0.073, 0.131)
	Breeding dispersal	75	**49**	352	652	0.109 (0.083, 0.135)

(Table 2). In this last case, the exponential distribution obtained the lower AIC (Table 2). The estimated scale parameter γ of Cauchy distribution was very similar among species and age classes (Table 2), i.e., increasing distance the probability of dispersal events decreased in a similar way. The normal distribution always resulted to be the worst model for our dispersal data (Table 2; Fig. 2).

Discussion

Although in both species most individuals were philopatric, dispersing birds were able to cross large distances, suggesting the capability to compensate for habitat fragmentation. We found a heavy-tailed distribution to be the best model for dispersal distances, consistently with similar previous studies (Paradis et al. 2002; Winkler et al. 2005; Van Houtan et al. 2007, 2010). The exception represented by natal dispersal of Moustached Warblers, where the exponential distribution was selected as the best model (Table 2), should be cautiously considered because of the low sample size. Long-distance dispersal cases are rare, thus reduced samples are likely to fail in detecting them. When loosing these events, heavy-tailed distributions, given their characteristics (see the “Introduction”), will result as less adequate to describe dispersal distances. Further investigation using additional data will be needed to assess if juvenile Moustached Warblers from our study populations are able to disperse over long distances. The good dispersal ability of Reed Warblers was also found in British populations (Paradis et al. 1998) and was confirmed by the low genetic differentiation among European populations (Kralj et al. 2010; Procházka et al. 2011; Ceresa et al. 2015). Our results about this species indicate that in our study population juveniles disperse more frequently than adults, but with similar probability of settling with increasing distance, which indicates that dispersal ability is not different among the two age classes.

The two species showed a similarly low tendency to disperse, possibly caused by the reduced opportunities of finding new breeding sites and/or by the high costs of dispersal (e.g., increased mortality, physiological costs;

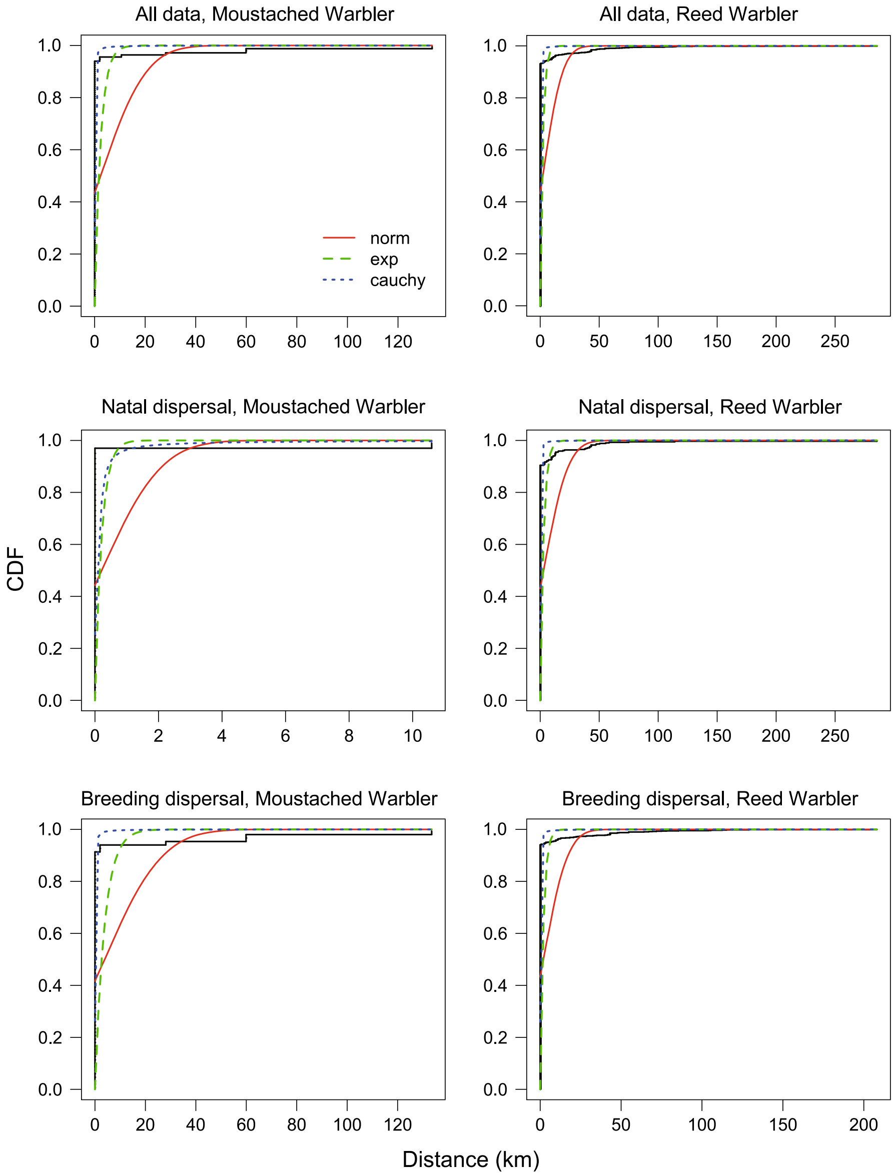

Fig. 2 Cumulative density functions (CDF) of the normal (norm), exponential (exp) and Cauchy (cauchy) distributions fitted to dispersal distances in Spanish populations of Moustached and Reed Warbler. The AIC values for the fitted distributions are provided in Table 2. The *black solid line* represents the CDF of the observed dispersal distances. The *x*-axis scales are different among the panels

Waser et al. 1994; Plissner and Gowaty 1996). At a marshland in Hungary, Vadász et al. (2008) found higher natal and breeding site fidelity in Moustached than in Reed Warblers, and in both species breeding site fidelity was higher than natal site fidelity. The results of Vadász et al. (2008) can not anyway be compared with ours, because in the first case the authors used returning rates at a single site (mortality and dispersal can not be distinguished), while we compared the proportion of recoveries obtained at and outside each ringing site. Ceresa et al. (2015) found that gene flow among Spanish populations of the two warblers, although occurring, was partly limited or even restricted. The high natal and breeding philopatry we found in both species may help to explain such limitation in gene flow, as hypothesized by Ceresa et al. (2015).

The lack of significant differences in the distribution of dispersal distances between the two species and the high similarity in the estimated Cauchy scale parameter

(Table 2) contrast with the hypothesis of higher dispersal ability in Reed Warblers (long-distance migrants) than in Moustached Warblers (resident/short distance migrants). Indirect information from genetic data indicated that Spanish populations of both species have a good dispersal capability, consistently with our results, but also showed lower population differentiation in Reed than in Moustached Warblers (Ceresa et al. 2015), indicating higher gene flow in the first species. Consistently with a limited gene flow, for Moustached Warblers we found no case of dispersal between the three populations considered in Ceresa et al. (2015), i.e., one small isolated inland population, one at the Mediterranean coast and one on the Mallorca Island. These populations are separated by the sea or by large land zones where the species does not breed. We found dispersal cases only between marshlands along the Mediterranean coast of Spain, the only area of the country where the breeding range is relatively continuous (see Castany and López 2006). Unfortunately, as far as we know there are no available genetic data to compare birds from different breeding sites along the coast. The Mallorca Island hosts the largest Spanish population of Moustached Warblers (approximately 500 breeding pairs) and has been the object of intense ringing activity (Castany 2003). The lack of recoveries from or towards this island is consistent with the restricted gene flow between the Mallorcan population and those of the mainland, which suggested that the sea represents an effective barrier for this species (Ceresa et al. 2015). The extinction of several bird species on Barro Colorado Island (Panama) showed that water can represent a barrier to immigration (Willis 1974; Robinson 1999), and other studies suggest that water bodies are more effective barriers than land areas of the same width, at least for some species (Hodges and Krementz 1996; Machtans et al. 1996). Unlike Moustached Warblers, Reed Warblers showed low populations differentiation even when the breeding sites were separated by the sea (Ceresa et al. 2015). According to the authors, the interspecific difference in population differentiation could be due to differences in dispersal ability, breeding site fidelity, capability of crossing the sea, population size or breeding range patchiness (Ceresa et al. 2015). Our results do not support the first two possible explanations, thus the lower population differentiation in Reed than in Moustached Warblers is more likely to be determined by the more continuous breeding range/larger population size (see the references in the "Introduction") and the higher capability of crossing stretches of sea of the first species. This comparison among ringing data and genetic information should be anyway cautiously considered. For example, differences in gene flow may also be influenced by possible interspecific differences in the fitness costs of dispersal, and we have no information about this issue. The similarity in dispersal patterns between the two species may be explained by their close phylogenetic relatedness (Sutherland et al. 2000). Furthermore, both warblers breed in a naturally patchy habitat, thus their dispersal ability may have evolved according to the same need of crossing more or less wide areas of unsuitable habitat to find new breeding sites.

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References

Banco de datos de anillamiento del remite ICONA – Ministerio de Medio Ambiente (2015) Datos de anillamiento y recuperaciones en España. Ministerio de Agricultura, Alimentación y Medio Ambiente, SEO/BirdLife, ICO, EBD-CSIC y GOB. Madrid (in Spanish)

Begon M, Townsend CR, Harper JL (2006) Ecology: from individual to ecosystems, 4th edn. Blackwell Publishing, Oxford

Blomqvist D, Fessl B, Hoi H, Kleindorfer S (2005) High frequency of extra-pair fertilisation in the moustached warbler, a songbird with a variable breeding system. Behaviour 142:1133–1148

Bohonak AJ (1999) Dispersal, gene flow, and population structure. Q Rev Biol 74:21–45

Burnham KP, Anderson DR (2002) Model selection and multi-model inference: a practical information-theoretic approach. Springer Verlag, New York

Cantos FJ, Tellería JL (1994) Stopover site fidelity of four migrant warblers in the Iberian Peninsula. J Avian Biol 25:131–134

Carrascal LM, Palomino D (2008) Las aves comunes reproductoras en España. Población en 2004–2006. SEO/BirdLife, Madrid (in Spanish with English abstract)

Carrascal LM, Weykam S, Palomino D, Lobo JM, Díaz L (2005) Atlas Virtual de las Aves Terrestres de España. http://www.vertebradosibericos.org/atlasaves.html. Accessed 16 Feb 2016

Castany J (2003) El carricerín real (*Acrocephalus melanopogon*) en el P. N. del Prat de Cabanes-Torreblanca. Doctoral thesis. University of Valencia, Valencia (in Spanish)

Castany J, López G (2006) El carricerín real en España. I Censo Nacional (2005). SEO/BirdLife, Madrid (in Spanish with English abstract)

Ceresa F, Belda EJ, Kvist L, Rguibi-Idrissi H, Monrós JS (2015) Does fragmentation of wetlands affect gene flow in sympatric *Acrocephalus* warblers with different migration strategies? J Avian Biol 46:577–588

Cooper NW, Murphy MT, Redmond LJ, Dolan AC (2009) Density-dependent age at first reproduction in the eastern kingbird. Oikos 118:413–419

Delignette-Muller ML, Dutang C (2015) fitdistrplus: An R Package for fitting distributions. J Stat Softw 64:1–34. http://www.jstatsoft.org/v64/i04/. Accessed 2 Sep 2015

Frankham R, Ballou JD, Briscoe DA (2010) Introduction to conservation genetics, 2nd edn. Cambridge University Press, Cambridge

Hengeveld R (1994) Small step invasion research. Trends Ecol Evol 9:339–342

Hodges MF Jr, Krementz DG (1996) Neotropical migratory breeding bird communities in riparian forests of different widths along the Altamaha River, Georgia. Wilson Bulletin 108:496–506

Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77:282–291

Kennerley P, Pearson D (2010) Reed and bush warblers. Christopher Helm Publishers Ltd., London

Koenig WD, Van Vuren D, Hooge PN (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends Ecol Evol 11:514–517

Kralj J, Procházka P, Fainová D, Patzenhauerová H, Tutiš V (2010) Intraspecific variation in the wing shape and genetic differentiation of reed warblers *Acrocephalus scirpaceus* in Croatia. Acta Ornithologica 45:51–58

Lambrechts MM, Blondel J, Caizergues A, Dias PC, Pradol R, Thomas DW (1999) Will estimates of lifetime recruitment of breeding offspring on small-scale study plots help us to quantify processes underlying adaptation? Oikos 86:147–151

Machtans CS, Villard MA, Hannon SJ (1996) Use of riparian buffer strips as movement corridors by forest birds. Conserv Biol 10:1366–1379

Nathan R, Perry G, Cronin JT, Strand AE, Cain ML (2003) Methods for estimating long-distance dispersal. Oikos 103:261–273

Newton I (1992) Experiments on the limitation of bird numbers by territorial behaviour. Biol Rev 67:129–173

Norberg UM (1990) Vertebrate flight, mechanics, physiology, morphology, ecology and evolution. Springer Verlag, Berlin

Paracuellos M, Tellería JL (2004) Factors affecting the distribution of a waterbird community: the role of habitat configuration and bird abundance. Waterbirds 27:446–453

Paradis E, Baillie SR, Sutherland WJ, Gregory RD (1998) Patterns of natal and breeding dispersal in birds. J Anim Ecol 67:518–536

Paradis E, Baillie SR, Sutherland WJ (2002) Modeling large-scale dispersal distances. Ecol Model 151:279–292

Peirò IG (2003) Intraspecific variation in the wing shape of the long-distance migrant Reed Warbler *Acrocephalus scirpaceus*: effects of age and distance of migration. Ardeola 50:31–37

Plissner JH, Gowaty PA (1996) Patterns of natal dispersal, turnover, and dispersal costs in eastern bluebirds. Anim Behav 51:1307–1322

Procházka P, Stokke BG, Jensen H, Fainová D, Bellinvia E, Fossøy F, Vikan JR, Bryja J, Soler M (2011) Low genetic differentiation among reed warbler *Acrocephalus scirpaceus* populations across Europe. J Avian Biol 42:103–113

R Core Team (2014) R: A language and environment for statistical computing. R foundation for statistical computing, Vienna

Robinson WD (1999) Long-term changes in the avifauna of Barro Colorado Island, Panama, a tropical forest isolate. Conserv Biol 13:85–97

SEO/BirdLife (2016a) Acrocephalus melanopogon. Anillamientos por década. http://www.anillamientoseo.org/. Accessed 19 Feb 2016 (in Spanish)

SEO/BirdLife (2016b) Acrocephalus scirpaceus. Anillamientos por década. http://www.anillamientoseo.org/. Accessed 19 Feb 2016 (in Spanish)

Shigesada N, Kawasaki K, Takeda Y (1995) Modeling stratified diffusion in biological invasions. Am Nat 146:229–251

Silva JP, Phillips L, Jones W, Eldridge J, O'Hara E (2007) Life and Europe's wetlands, restoring a vital ecosystem. Office for Official Publications of the European Communities, Luxembourg

Sutherland GD, Harestad AS, Price K, Lertzman KP (2000) Scaling of natal dispersal distances in terrestrial birds and mammals. Conservation ecology 4:16. http://www.consecol.org/vol4/iss1/art16. Accessed 23 Oct 2015

Vadász C, Német Á, Karcza Z, Loránt M, Biró C, Csörgő T (2008) Study on breeding site fidelity of *Acrocephalus* Warblers in Central Hungary. Acta Zool Acad Sci H 54(Suppl. 1):167–175

Van Houtan KS, Pimm SL, Halley JM, Bierregaard RO Jr, Lovejoy TE (2007) Dispersal of Amazonian birds in continuous and fragmented forest. Ecol Lett 10:219–229

Van Houtan KS, Bass OL Jr, Lockwood J, Pimm SL (2010) Importance of estimating dispersal for endangered bird management. Conservation Letters 3:260–266

Van Vessem J, Hecker N, Tucker GM (1997) Inland wetlands. In: Tucker GM, Evans MI (eds) Habitats for birds in Europe: A conservation strategy for the wider environment. BirdLife Conservation Series 6. BirdLife International, Cambridge, pp 125–158

Waser PM, Creel SR, Lucas JR (1994) Death and disappearance: estimating mortality risk associated with philopatry and dispersal. Behav Ecol 5:135–141

Willis EO (1974) Populations and local extinctions of birds on Barro Colorado Island, Panama. Ecol Monogr 44:153–169

Winkler DW, Wrege PH, Allen PE, Kast TL, Senesac P, Wasson MF, Llambías PE, Ferretti V, Sullivan PJ (2004) Breeding dispersal and philopatry in the tree swallow. Condor 106:768–776

Winkler DW, Wrege PH, Allen PE, Kast TL, Senesac P, Wasson MF, Sullivan PJ (2005) The natal dispersal of tree swallows in a continuous mainland environment. J Anim Ecol 74:1080–1090

General discussion and future perspectives

We have provided new information about the breeding ecology of two *Acrocephalus* warblers and their niche overlap in sympatric conditions. Our results (chapter 2) provided insights about possible competitive interactions between our study species, and about the possible influence of such interactions in determining the early breeding of the moustached warbler, which is peculiar among *Acrocephalus* warblers breeding in Europe. Although we could not clearly assess the role of exploitative competition for food (a common problem when using observational data; Begon et al. 2006), our study suggests that it could play a more important role than previously hypothesized within assemblages of reedbed-nesting passerines. Future studies may obtain clearer results about competitive interactions by taking into account more breeding sites to compare the niche dimensions of wetland passerines in sympatry and allopatry areas. In addition, considering whole assemblages of co-occurring wetland passerines instead of pairs of species may allow to obtain more complete information.

Among European passerines, the temporal dynamic of food availability and food demand has been rarely documented for wetland species (Bibby & Thomas 1985), while most of such information has been obtained for some woodland passerines (e.g. Thomas et al. 2001, Cresswell & McCleery 2003, Both et al. 2006, Visser et al. 2006, Veen et al. 2010; but see also Hussel 1972 and Bryant 1975 about Emberizidae and house martin *Delichon urbicum*). The trophic resources of these species during the breeding season are represented substantially by caterpillars, which typically show a clear peak of seasonal abundance, thus breeding birds need to match the nestling rearing period with such peak. In the case of our study species, given their varied diet the different phenologies of the several prey types produced an overall increase of food availability throughout the breeding season (chapter 2), rather than a clear peak, as found also by Bibby & Thomas (1985) for reed warblers in England. For this reason, Halupka et al. (2008) concluded that the advancement of breeding phenology in a reed warbler population was probably not connected to possible shifts of food availability. A further development of our work will consist in examining food need and availability across more years, looking for possible interannual differences and for the relationships among breeding phenologies, trophic resources and climatic variables.

Our results about the effects of the emetic used to obtain food samples (chapter 1) may help researchers in finding the most appropriate food sampling technique. In particular, we hope to have encouraged investigators to abandon the use of clearly harmful substances on passerines, such as antimony potassium tartrate. In any case, given the interspecific differences in the effects of emetics, future uses of apomorphine on other bird species should include a rigorous assessment of its effects.

The results reported in chapter 3 represent the first assessment of genetic population structure and demographic history for the moustached warbler and contribute to a better knowledge of Iberian and Moroccan populations of the reed warbler, also confirming the good dispersal capability of this species reported by previous studies (Kralj et al. 2010, Procházka et al. 2011). For both warblers we found evidence for gene flow among distant breeding areas, although the sea seems to represent an effective dispersal barrier for moustached warblers, possibly because of the risks connected with crossing large water bodies. Most of SW European moustached warbler population breeds along the Mediterranean coast, across a highly scattered range. Supplementary data from more breeding sites may clarify the degree of gene flow within this area, which includes the large population breeding in S France (2000-6000 pairs, BirdLife International 2004), which partly winters in Spain (Castany 2003). At the moment, mark-recapture data (chapter 4) indicate the exchange of individuals at least among the Spanish Mediterranean coastal marshlands. Although the comparison between the genetic results and mark-recapture data should be cautiously considered, we found an interesting overall consistency in indicating the good dispersal ability of the two study species and the role of the sea as a dispersal barrier for moustached warblers. This comparison also provided insights about how to interpret the differences in genetic population differentiation found between moustached and reed warblers. In fact, the similar distributions of dispersal distances and philopatry between the two species (chapter 4) suggest that other characteristics (such as population size, breeding range patchiness and the capability of crossing natural barriers) are more likely to be involved in determining the differences found through genetic analysis. Comparing results obtained from both mark-recapture and genetic data allowed us to obtain a better understanding of dispersal patterns and genetic population structure (see also Hansson et al 2000, 2002).

Large ringing databases have been often used to investigate bird migration, but large scale studies about dispersal based on this kind of data are relatively scarce (Paradis et al 1998, 2002, Van Houtan et al. 2007). Such studies and our results confirmed that valuable information about dispersal can be obtained from ringing data. Additional similar investigations may help to clarify the general dispersal patterns of birds explored by Paradis et al. (1998) and Sutherland et al. (2000). However, an effort to obtain more reliable results will consist in taking into account the spatial variation in capture effort, like in Van Houtan et al. (2010). This could be challenging when considering very large areas as in our study, due to the necessity of detailed data about field effort and species' distribution. In addition, given the relative rarity of long-distance dispersal events, large numbers of recaptures are required to avoid missing such relevant cases. Indeed, in our study (chapter 4), the scarcity of recaptures for young moustached warblers did not allow us to assess if such birds are actually not able to disperse over long distances. Future additional data will hopefully provide sufficient information to clarify this issue. Heavy-tailed distributions resulted to be the most adequate models to describe dispersal distances (see also Paradis et al. 2002, Winkler et al. 2005, Van Houtan et al. 2007, 2010), indicating higher frequency of long-distance dispersal events than expected in case of constant probability of settling. Such result has been found in a variety of morphologically and ecologically very different passerine species (blue tit *Cyanistes cearuleus* and great tit *Parus major* in Paradis et al. 2002; tree swallow *Tachycineta bicolor* in Winkler et al. 2005; Tyrannidae, Thamnophilidae and Furnariidae in Van Houtan et al. 2007; seaside sparrow *Ammodramus maritimus* in Van Houtan et al. 2010), suggesting that it could represent a common pattern, at least among passerine birds.

References

Begon M., Townsend C. R. & Harper J. L. 2006. *Ecology: from individual to ecosystems*, 4th ed. Blackwell Publishing.

BirdLife International. 2004. *Birds in Europe: population estimates, trends and conservation status*. BirdLife International.

Both C., Bouwhuis S., Lessells C. M. & Visser M. E. 2006. Climate change and population declines in a long-distance migratory bird. Nature 441: 81-83.

Bryant D. M. 1975. Breeding biology of House Martins (*Delichon urbica*) in relation to aerial insect abundance. Ibis 117: 180-216.

Castany J. 2003. El carricerín real (*Acrocephalus melanopogon*) en el P. N. del Prat de Cabanes-Torreblanca. Doctoral thesis. University of Valencia.

Catchpole C. K. 1978. Interspecific territorialism and competition in *Acrocephalus* warblers as revealed by playback experiments in areas of sympatry and allopatry. Animal Behaviour 26: 1072-1080.

Cresswell W. & McCleery R. 2003. How great tits maintain synchronization of their hatch date with food supply in response to long-term variability in temperature. Journal of Animal Ecology 72: 356-366.

Halupka L., Dyrcz A. & Borowiec M. 2008. Climate change affects breeding of reed warblers *Acrocephalus scirpaceus*. Journal of Avian Biology 39: 95-100.

Hansson B., Bensch S., Hasselquist D., Lillandt B. G., Wennerberg L. & Von Schantz T. 2000. Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNS fingerprinting. Molecular Ecology 9: 1529-1538.

Hansson B., Bensch S., Hasselquist D. & Nielsen, B. 2002. Restricted dispersal in a long-distance migrant bird with patchy distribution, the great reed warbler. Oecologia 130: 536-542.

Hoi H., Eichler T. & Dittami, J. 1991. Territorial spacing and interspecific competition in three species of reed warblers. Oecologia 87: 443-448.

Honza M., Moksnes A., Roskraft E. & Øien I.J. 1999. Effect of great reed warbler Acrocephalus arundinaceus on the reproductive tactics of the reed warbler A. scirpaceus. Ibis 141: 489-493.

Hussel D. J. T. 1972. Factors affecting clutch size in arctic passerines. Ecological Monographs 42: 317-364.

Kralj J., Procházka P., Fainová D., Patzenhauerová H. & Tutiš V. 2010. Intraspecific variation in the wing shape and genetic differentiation of reed warblers *Acrocephalus scirpaceus* in Croatia. Acta Ornithologica 45: 51-58.

Paradis E., Baillie S. R., Sutherland W.J. & Gregory R.D. 1998. Patterns of natal and breeding dispersal in birds. Journal of Animal Ecology 67: 518-536.

Paradis E., Baillie S. R. & Sutherland W. J. 2002. Modeling large-scale dispersal distances. Ecological Modelling 151: 279-292.

Procházka P., Stokke B. G., Jensen H., Fainová D., Bellinvia E., Fossøy F., Vikan J. R., Bryja J. & Soler M. 2011. Low genetic differentiation among reed warbler *Acrocephalus scirpaceus* populations across Europe. Journal of Avian Biology 42: 103-113.

Sutherland G. D., Harestad A. S., Price K. & Lertzman K. P. 2000. Scaling of natal dispersal distances in terrestrial birds and mammals. Conservation Ecology 4:16 URL http://www.consecol.org/vol4/iss1/art16.

Thomas D. W., Blondel J., Perret P., Lambrechts M. M. & Speakman J. R. 2001. Energetic and fitness costs of mismatching resource supply and demand in seasonally breeding birds. Science 291: 2598-2600.

Van Houtan K. S., Pimm S. L., Halley J. M., Bierregaard R. O. Jr & Lovejoy T. E. 2007. Dispersal of Amazonian birds in continuous and fragmented forest. Ecology Letters 10: 219-229.

Van Houtan K. S., Bass O. L. Jr, Lockwood J. & Pimm S. L. 2010. Importance of estimating dispersal for endangered bird management. Conservation Letters 3: 260-266.

Veen T., Sheldon B. C., Weissing F. J., Visser M. E. Qvarnström A & Sætre G. P. 2010. Temporal differences in food abundance promote coexistence between two congeneric passerines. Oecologia 162: 873-884.

Visser M. E., Hollemann L. J. M. & Gienapp P. 2006. Shift in caterpillar biomass phenology due to climate change and its impact on the breeding ecology of an insectivorous bird. Oecologia 147: 167-172.

Winkler D. W., Wrege P. H., Allen P. E., Kast T. L., Senesac P., Wasson M. F. & Sullivan P. J. 2005. The natal dispersal of tree swallows in a continuous mainland environment. Journal of Animal Ecology 74:1080-1090.

Conclusions

1. We found support for the high safeness and effectiveness of apomorphine, a scarcely used emetic substance. Such information will hopefully help researchers to choose the best food sampling technique, in order to reduce the impact of diet studies on birds.
2. Exploitative competition has been scarcely taken into account in studies on coexisting *Acrocephalus* warblers. At a sympatry breeding site, we assessed that the moustached and the reed warbler are potential competitors for food, given their high diet overlap. This may help to explain their reduced temporal and spatial overlap observed at the study site.
3. We described the temporal dynamic of food availability and food demand for the two study species during the breeding season. The period of higher food demand for reed warblers coincided with high food availability and high abundance of potential competitors, while most moustached warblers reared their chicks earlier, when both food availability and abundance of potential competitors were lower.
4. Within the reedbed invertebrate community, both warblers clearly selected their preys, preferring spiders, beetles and hymenopterans while avoiding dipterans and hemipterans.
5. In both the study species we found evidences of gene flow among sampling sites, suggesting that these two warblers are able to compensate for habitat fragmentation. However, in both species gene flow was partly restricted, possibly because of the large distances among sampling sites, breeding site fidelity, or due to geographical barriers.
6. The lower genetic population differentiation in reed than in moustached warblers may be due to possible interspecific differences in dispersal ability, philopatry and capability in crossing natural barriers, as well as to the larger population size and the more continuous breeding range of the first species. The analysis of mark-recapture data, however, did not show significant differences in dispersal ability or philopatry between the two warblers. The sea is likely to represent an effective dispersal barrier for moustached warblers, but probably not for reed

warblers, given the low differentiation found between Moroccan and Iberian individuals of this last species.

7. Consistently with previous studies on wetland passerines (Hansson et al. 2008, Neto et al. 2012, Arbabi et al. 2014), we found evidence for postglacial population growth in both study species, but such increasing and the colonisation of new areas occurred in partly different ways between the two warblers.

8. Mark-recapture data confirmed the good dispersal ability of moustached and reed warblers, although most individuals were philopatric. We found no evidence for differences in dispersal ability between adults and young in both warblers, while among reed warblers young birds dispersed more frequently than adults.

9. The similar distribution of dispersal distances between the moustached and the reed warbler does not support the hypothesis of higher dispersal ability in long distance than in sedentary/short distance migrant birds suggested by previous studies (Paradis et al. 1998, Sutherland et al. 2000). Such similarity may be due to the phylogenetic relatedness between the two warblers and to similar evolutionary pressures.

10. Among a set of dispersal models, a heavy-tailed distribution resulted to be the best in describing dispersal distances, indicating higher probability of long-distance dispersal events than expected if probability of settling were constant. This is consistent with previous studies concerning some ecologically and morphologically very different passerine species (Paradis et al. 2002, Winkler et al. 2005, Van Houtan et al. 2007, 2010). This suggests that such distribution of dispersal distances is a common pattern, at least among passerine birds.

Resumen ampliado

Acrocephalus y *Locustella* son dos géneros de aves paseriformes típicos de ambientes húmedos. Se trata de aves de pequeño tamaño (~10-30 g), mayoritariamente de colores apagados e insectívoras. En Europa, especies de estos dos géneros constituyen la mayor parte de los ensamblajes de paseriformes en los carrizales. Como consecuencia, en muchos carrizales coexisten varias especies similares a nivel ecológico y morfológico. Para que esta coexistencia sea posible, se puede esperar que existan diferencias a nivel de nicho ecológico entre estas especies, y efectivamente en algunos estudios se encontraron diferencias en las preferencias ambientales (Hoi et al. 1991, Poulin et al. 2002). El solapamiento de nichos tróficos y la posibilidad de competencia por el alimento entre paseriformes palustres han sido poco estudiados, probablemente porque en un hábitat muy productivo como los carrizales se asumió una disponibilidad de comida demasiado elevada como para determinar interacciones competitivas. Sin embargo, la disponibilidad de alimento puede influir en la abundancia de paseriformes palustres (Poulin et al. 2002), y esto sugiere que los recursos tróficos que están a disposición de estas especies no son siempre sobreabundantes.

Además de ser ambientes muy productivos, los carrizales son también hábitats muy fragmentados, tanto por causas naturales como por el resultado de actividades humanas (Finlayson et al. 1992, Van Vessem et al. 1997, Silva et al. 2007). Es en estas condiciones es cuando resulta crucial la capacidad de dispersión de la especies que los habitan, para garantizar un suficiente flujo genético entre poblaciones (Frankham et al. 2010). Las mayoría de las aves tiene una elevada capacidad de dispersión (Koenig et al. 1996), pero existen importantes diferencias interespecificas, relacionadas de manera compleja y aún con poca claridad entre varias características especificas (como la estrategia migratoria, tamaño poblacional, rango de distribución y preferencias ambientales) y con la relación filogenética entre especies (Paradis et al. 1998, Sutherland et al. 2000). Datos y estudios comparativos adicionales son necesarios para mejorar el conocimiento sobre la dispersión de las aves.

En la presente tesis hemos considerado el carricerín real *Acrocephalus melanopogon* y el carricero común *Acrocephalus scirpaceus*. Estudiar estos dos *Acrocephalus* representa una oportunidad para comparar dos especies filogenética y estrictamente relacionadas, y además muy similares en tamaño (~10 g), pero con importantes diferencias a nivel ecológico y etológico (descritas en los párrafos siguientes). El objetivo de esta tesis fue de estudiar algunos aspectos de la ecología reproductiva, la capacidad de dispersión y la estructura genética de las poblaciones de estas dos especies en un contexto de elevada fragmentación de hábitat, y su solapamiento de nicho en condiciones de simpatría; así como comparar los resultados obtenidos para las dos especies teniendo en cuenta sus diferencias a nivel ecológico y etológico. Además, en un trabajo metodológico sobre los efectos de un emético (apomorfina) en paseriformes palustres (capítulo 1), también hemos considerado otras dos especies, la buscarla unicolor *Locustella luscinioides* y el carricero tordal *Acrocephalus arundinaceus*.

El carricerín real se reproduce principalmente en Europa y Asia, a lo largo de un rango de distribución discontinuo entre el suroeste de Europa y Kazakstán; también cría en algunas áreas muy localizadas en el norte de África (Kennerley & Pearson 2010). La población española pertenece a la subespecie nominal, y se estima que está formada aproximadamente por unas 1000 parejas, y parece haber sufrido un claro declive en las últimas décadas (Castany & López 2006). Se reproduce, casi siempre en simpatría con el más abundante carricero común, en humedales de la costa Mediterránea, en las Islas Baleares y, con pequeñas poblaciones, en algunas zonas de Extremadura, Castilla-La Mancha y Aragón (Castany & López 2006). En Europa, es el único *Acrocephalus* cuyas poblaciones son sedentarias o migran a corta distancia (Kennerley & Pearson 2010). Las preferencias de hábitat del carricerín real son especializadas, ya que para criar necesita carrizales inundados donde además de carrizo *Phragmites australis* se encuentren también otras plantas de los géneros *Typha*, *Scirpus*, *Cladium* y *Juncus* (Castany 2003, Kennerley & Pearson 2010). Como consecuencia de tal especialización, esta especie puede resultar perjudicada por prácticas de gestión de carrizales, tales como el corte invernal o variaciones del nivel hídrico, que pueden reducir la disponibilidad de lugares de cría o de alimento (Poulin et al. 2002, Schmidt et al. 2005). El carricerín real puede alimentarse de muchos tipos de insectos y sus larvas, de arañas, de isópodos, de pequeños caracoles dulceacuícolas y, ocasionalmente, de frutos (Dyrcz 2016). La reproducción comienza antes que en los demás *Acrocephalus* europeos; en el área Mediterránea la puesta de huevos puede tener lugar ya en marzo (Castany 2003, Kennerley & Pearson 2010). En la subespecie nominal, tanto los adultos como los jóvenes mudan completamente el plumaje a finales de verano/inicio del otoño; también efectúan una muda pre-reproductiva parcial (Kennerley & Pearson 2010).

El carricero común es una especie con un amplio rango de distribución en Europa, además de criar también en amplias zonas de Asia y de África. En España, la población reproductora ha sido estimada entre 20000 y 100000 parejas (BirdLife International 2004), las cuales se concentran especialmente en los valles del Ebro, Tajo y Guadalquivir, a lo largo de la costa Mediterránea y en la Meseta Norte (Gainzarain 2003). Las poblaciones europeas de esta especie son migrantes de larga distancia e invernan en el África sub-Sahariana (Kennerley & Pearson 2010). Estudios genéticos han evidenciado una baja diferenciación entre poblaciones europeas, algo que indica una elevada capacidad de dispersión (Kralj et al. 2010, Procházka et al. 2011). Datos de marcaje y recaptura colectados en poblaciones británicas han confirmado esta capacidad, con distancias de dispersión de hasta 300 km (Paradis et al. 1998). Entre los *Acrocephalus*, el carricero común es una especie muy adaptable, ya que puede criar en carrizales de diverso tipo, incluidos los muy pequeños o sin agua, aun que prefiere carrizales inundados (Kennerley & Pearson 2010). Su dieta incluye insectos y sus larvas, arañas, opiliones, pseudoescorpiones, pequeños moluscos y, con menor frecuencia, frutos, flores y semillas (Dyrcz et al. 2016). En la subespecie nominal, tanto los adultos como los jóvenes efectúan una muda parcial a finales de verano y una muda completa en las zonas de invernada, aun que en poblaciones Ibéricas se han encontrado casos de muda completa en las zonas de reproducción (Kennerley & Pearson 2010).

En el capítulo 2 presentamos los resultados de un trabajo llevado a cabo en el año 2012 en un humedal donde el carricerín real y el carricero común crían en simpatría, el Marjal de Pego-Oliva (Comunidad Valenciana, España). Nuestro objetivo fue evaluar el solapamiento de nichos entre ellas a nivel trófico, temporal y espacial. Con este propósito, hemos colectado información sobre la composición de la dieta, la disponibilidad de alimento, la fenología reproductiva y la distribución espacial a nivel local de las dos especies. Para describir la dieta, hemos obtenido muestras de comida regurgitada al suministrar a las aves un emético, y clasificado las presas presentes en las muestras hasta el nivel de Orden. Para medir la disponibilidad de alimento hemos llevado a cabo muestreos estandarizados de invertebrados, que posteriormente fueron clasificados hasta el nivel de Orden. La fenología reproductiva se estudió a través de anillamiento intensivo estandarizado, colectando también datos detallados sobre el desarrollo de la placa incubatriz de las hembras; teniendo en cuenta el tiempo necesario para la incubación de los huevos, a partir de los datos de placa incubatriz, hemos individuado el periodo donde más parejas alimentaban a sus pollos en el nido, y lo hemos considerado como el periodo de máxima demanda de alimento (Van Noordwijk et al. 1995). La distribución de las dos especies en el humedal se comprobó a través de puntos de escucha. A partir de estos datos, hemos averiguado que en este lugar de cría las dos especies tienen un amplio solapamiento de nicho trófico, siendo así potenciales competidoras por la comida. En ambas especies, coleópteros y himenópteros fueron las presas más frecuentes, representando juntos más del 70% de la dieta. La selección de presas resultó ser similar, ya que tanto el carricerín real como el carricero común seleccionaron positivamente arañas, coleópteros y himenópteros, y negativamente dípteros y hemípteros. En la dieta no se encontraron algunas categorías de invertebrados que fueron muestreadas en el carrizal (larvas de insectos, huevos, Gasteropoda, Glomerida, Lepidoptera, Odonata, Parasitiformes, Thysanoptera), pero sus proporciones en la dieta y el medio ambiente no resultaron significativamente diferentes, así que no se puede afirmar que fueron seleccionadas negativamente. A diferencia de lo observado a nivel trófico, las dos especies diferían tanto en distribución espacial (< 50% de solapamiento) como en fenologías reproductivas. La disponibilidad de alimento aumentó, con fluctuaciones, a lo largo de la estación reproductiva, alcanzado el máximo a finales de junio, mientras que los periodos de máxima demanda de alimento fueron en abril para los carricerínes reales y aproximadamente entre el 5 y el 25 de junio para los carriceros comunes. También la abundancia global de las dos especies, y consecuentemente de los posibles competidores, aumentó durante la estación reproductiva, resultando ser menor durante la máxima necesidad de alimento de los carricerínes reales en comparación con los carriceros comunes. Las diferencias en nichos temporales y espaciales encontradas entre las dos especies podrían representar una respuesta a la competencia interespecifica por la comida, donde el carricerín real reduce la competencia con el carricero común criando notablemente antes, y la segunda especie se establece preferiblemente en áreas no previamente ocupadas por la primera. Esto ayudaría también a explicar la peculiar fenología reproductiva del carricerín real, que es única entre los *Acrocephalus* europeos. De todas maneras, el papel de las interacciones competitivas no se puede aclarar a partir de nuestros resultados, y no pudimos comprobar si en algún momento la disponibilidad de alimento resultó ser escasa en comparación con la necesidad de las aves. Información más exhaustiva podría dar luz a estas cuestiones en futuros estudios sobre los nichos ecológicos de las dos especies en áreas de simpatría y alopatría. Así, en trabajos posteriores vamos a comparar disponibilidad y necesidad de alimento durante varios años, para investigar las posibles diferencias interanuales y las relaciones entre fenologías reproductivas, recursos tróficos y variables climáticas.

Para obtener las muestras de comida regurgitada se utilizó, como hemos mencionado, un emético. Hemos utilizado la apomorfina (un opiáceo), ya que ha sido descrita en la literatura como un emético eficaz y no dañino por las aves tratadas, al menos durante e inmediatamente después de la administración (Díaz 1989, Valera et al. 1997, Poulin et al. 2002), aunque sobre efectos posteriores solo existía información obtenidas de algunas aves en cautividad (paseriformes granívoros; Valera et al. 1997). Después de comprobar durante la actividad de campo la falta de mortalidad durante el tratamiento, hemos realizado un estudio para averiguar los efectos de la apomorfina sobre las aves después del tratamiento (capítulo 1). Con este propósito, hemos analizado datos de captura-recaptura de aves tratadas y no tratadas (carricerínes reales y carriceros comunes), para comparar su probabilidad de supervivencia en un periodo de 21 días después del tratamiento/primera captura. Las probabilidades de supervivencia y de recaptura fueron calculadas a través de modelos Cormack-Jolly-Seber. Además, comparamos la diferencia de masa entre recapturas de carricerínes reales tratados y no tratados, para averiguar si las aves tratadas, aun que vivas, habían sufrido un empeoramiento de sus condiciones. También medimos el nivel de eficacia del emético en todas las especies tratadas (incluyendo buscarla unicolor y carricero tordal), y comprobado si el momento del día o el tiempo transcurrido desde la preparación del emético influía en su eficacia. No hemos encontrado evidencias de efectos negativos de la apomorfina en el periodo considerado, ni en la probabilidad de supervivencia ni en la variación de masa. El emético resultó ser eficaz, ya que obtuvimos muestras de comida del 76.7% de las aves tratadas, pero con diferencias significativas entre especies. Ni la hora del día ni el tiempo desde la preparación del emético influía en su efectividad. Con este estudio hemos contribuido a mejorar el conocimiento sobre un emético hasta ahora escasamente utilizado, información que puede resultar útil en otras investigaciones a la hora de seleccionar el método de muestreo más adecuado. Por ejemplo, podría convencer a otros investigadores para abandonar el uso de eméticos más dañinos para paseriformes, como el tartrato de potasio. En cualquier caso, ya que los eméticos tienen efectos diferentes según la especie tratada, en futuros muestreos con apomorfina llevados a cabo sobre otras especies, va a ser necesario seguir recogiendo información para comprobar los efectos de la apomorfina durante y después del tratamiento.

Como ya he mencionado, los humedales son hábitats fragmentados por razones naturales y antrópicas. La destrucción y degradación de estos hábitats como consecuencia de las actividades humanas ha sido especialmente fuerte en el área Mediterránea (Silva et al. 2007). Para especies que viven en hábitats fragmentados, la capacidad de dispersión es especialmente importante para poder mantener un flujo genético suficiente entre poblaciones. Poblaciones aisladas y pequeñas tienen más probabilidades de incurrir en endogamia y en la deriva genética, y consecuentemente se enfrentan a mayores riesgos de extinción (Young & Clarke 2000, Frankham et al. 2010). En el capítulo 3, hemos investigado la estructura y la diversidad genética, así como la historia demográfica de poblaciones reproductoras de carricerín real y carricero común en contextos de elevada fragmentación de su hábitat, en España y Marruecos. Nuestros objetivos fueron contribuir a mejorar la información existente sobre las dos especies, comprobar los posibles efectos de la fragmentación de hábitat sobre las poblaciones consideradas y comparar los resultados obtenidos para las dos especies, considerando sus diferencias en estrategia migratoria, tamaño poblacional y discontinuidad del rango de distribución. Para el carricerín real, se trata del primer estudio sobre estructura genética de sus poblaciones e historia demográfica. Durante las estaciones reproductivas de 2012 y 2013 se colectaron muestras de sangre de las dos especies en el Marjal de Pego-Oliva, en Villafranca de los Caballeros y en S'Albufera de Mallorca, y (solo del carricero común) en Larache (Marruecos). A partir de las muestras (54 de carricerín real y 68 de carricero común) hemos obtenido información de microsatélites (16 loci) y de ADN mitocondrial (secuencias de citocromo oxidasa COI). Para el carricerín real hemos encontrado evidencias de flujo genético entre los sitios de muestreo, a pesar de la gran distancia que los separan (290-960 km), aunque dicho flujo resultó ser “parcialmente limitado” entre la costa Mediterránea (Pego-Oliva) y el interior de la Península (Villafranca), y “reducido” entre Mallorca y los humedales peninsulares. En el carricero común hemos encontrado una menor diferenciación genética entre sitios de muestreo que en el carricerín real, aun que el flujo genético resultó ser “parcialmente limitado”. En ninguna de las dos especies se obtuvieron evidencias claras de aislamiento por distancia. La diversidad haplotípica y nucleotídica resultaron ser mayores en el carricero común que en el carricerín real. La historia demográfica de las dos especies resultó ser parcialmente diferente, aunque en ambos casos encontramos evidencias de un incremento post-glacial del tamaño de población. En el carricero común hubo una divergencia genética hace 40000-47000 años, seguida por una mezcla aproximadamente hace 14000-17000 años. En el carricerín real, los análisis indican una divergencia entre la población peninsular y la de Mallorca hace aproximadamente 690 años, algo que indica que la isla fue colonizada o recolonizada en tiempos recientes. Los resultados mostraron que ambas especies tienen una buena capacidad de dispersión. Las limitaciones parciales al flujo genético podrían explicarse o bien por una alta filopatría, o por el efecto de barreras geográficas, o por la elevada distancia entre las áreas de muestreo (aunque los resultados sobre aislamiento por distancia no apoyan claramente esta última posibilidad). La menor diferenciación encontrada en los carriceros comunes puede ser debida a una mayor capacidad de dispersión de la especie, a un rango de distribución más continuo en el área de estudio, a un mayor tamaño de población, a una menor filopatría o a una mayor capacidad de cruzar barreras geográficas en comparación con los carricerínes reales. De hecho, para esa última especie el mar que separa la Península Ibérica de la isla de Mallorca (~200 km) parece constituir una barrera eficaz; hay que tener en cuenta que cruzar el mar es probablemente algo insólito para individuos de las poblaciones estudiadas de carricerín real, ya que en su mayoría son sedentarios o migran sin cruzar el Mediterráneo (Castany 2003), mientras que para los carriceros comunes se trata de una capacidad necesaria para llegar a las áreas de invernada en África. Algunos estudios previos ya sugirieron que, para algunas especies, los cuerpos de agua pueden representar barreras más eficaces que áreas de tierra de la misma extensión (Hodges & Krementz 1996, Machtans et al. 1996). La mayor diversidad haplotípica en el carricero común podría ser debida a una mayor llegada de individuos en dispersión desde otras poblaciones, al mayor tamaño de población o a la mezcla ocurrida entre diferentes linajes. El incremento post-glacial del tamaño de la población ocurrido en ambas especies es consistente con estudios previos sobre otros paseriformes palustres, que durante el calentamiento global expandieron su rango de distribución a partir de sus refugios glaciales (Hansson et al. 2008, Neto et al. 2012).

Después de obtener información sobre la capacidad de dispersión de las especies estudiadas a partir de datos genéticos (capítulo 3), hemos seguido investigado utilizando también datos de marcaje y recaptura, para poder estudiar las distancias de dispersión (capítulo 4). Obtener información directa sobre las distancias de dispersión es especialmente difícil en organismos con una elevada capacidad de movimiento como las aves, ya que es necesario considerar áreas de estudio muy amplias para poder detectar los casos de dispersión a larga distancia. Estos casos son importantes ya que pueden influir la dinámica de las poblaciones y sus estructuras genéticas (Ibrahim et al. 1996, Nathan et al. 2003). Las grandes bases de datos de anillamiento de aves incluyen datos colectados sin los límites espaciales de una única área de estudio, y por esto pueden proporcionar información valiosa sobre la dispersión (Paradis et al. 1998, 2002). En el capítulo 4, hemos investigado los patrones de dispersión de poblaciones españolas de carricerín real y carricero común a partir de datos proporcionados por el Banco de datos de anillamiento del remite ICONA - Ministerio de Medio Ambiente. Esos datos consistían en todas las recapturas de individuos de las dos especies, que fueron anillados en España entre el año 1962 y el 2013. Nuestros objetivos fueron obtener información sobre las distancias de dispersión y la filopatría en las poblaciones consideradas, y averiguar la ocurrencia de posibles diferencias intraespecificas (entre jóvenes y adultos) e interespecificas. Además, entre tres modelos con claro significado biológico, queríamos encontrar el más adecuado para describir la dispersión en las poblaciones estudiadas. Como modelos candidatos hemos considerado la distribución normal, la distribución exponencial y la distribución Cauchy. El primer modelo es el más adecuado para describir movimientos lentos de difusión influenciados por varios factores estocásticos; en el segundo, al aumentar la distancia la probabilidad de establecimiento es constante; el tercer modelo predice eventos de dispersión de larga distancia más frecuentemente que en la distribución exponencial (Paradis et al. 2002, Van Houtan et al. 2007). Hemos considerado solo los individuos marcados durante la estación reproductiva y recapturados en años siguientes durante también la estación reproductiva. Para evitar incluir aves en migración en los análisis, hemos descartado las recapturas de larga distancia si se producían a lo largo del principal eje de migración en la Península Ibérica (NE-SO). No hemos encontrado evidencias de diferencias intra- o interespecificas en la distribución de las distancias de dispersión. La mayoría de las recapturas (> 90 % en ambas especies) se producían en el mismo sitio de marcaje, indicando un comportamiento filopátrico; además, no había deferencias entre carricerínes reales jóvenes y adultos, mientras que entre los carriceros comunes los adultos resultaron ser más filopátricos que los jóvenes. Aun que relativamente pocos, los individuos que se dispersaron se desplazaron en algunos casos a largas distancias, de hasta más de 100 km en ambas especies. La distribución Cauchy resultó ser la más adecuada para describir las distancias de dispersión en todos los casos, exceptuados los jóvenes carricerines reales donde el modelo exponencial resultó ser el mejor. Para esta categoría era la que teníamos el tamaño de muestra más pequeño (N = 50), y fue la única en que no encontramos casos de dispersión de larga distancia. El parámetro escala γ de la distribución Cauchy resultó ser muy similar entre especies y clases de edad, indicando que al aumentar de la distancia, la probabilidad de establecimiento baja de manera muy similar. La distribución normal representó en todos los casos el peor modelo de dispersión. Nuestros resultados sobre los modelos de dispersión son consistentes con estudios previos realizados en otros paseriformes, donde distribuciones con cola larga resultaron ser los más adecuados para describir las distancias de dispersión (Paradis et al. 2002, Winkler et al. 2005, Van Houtan et al. 2007, 2010). Estos estudios, y el nuestro, consideraron varias especies de paseriformes con características ecológicas y morfológicas muy diferentes, por lo que este tipo de distribución de las distancias de dispersión podría constituir un patrón común entre los paseriformes. En nuestros resultados, la excepción representada por los jóvenes carricerínes reales tiene que ser considerada con cautela dado el reducido tamaño de muestra, pues la baja proporción de individuos que se dispersan a una larga distancia evidencia la necesidad de disponer de un gran número de recapturas para detectar estos casos. Creemos que en futuros estudios, la obtención de datos adicionales podría aclarar si los jóvenes de esta especie tienen la capacidad de dispersarse a una larga distancia. La baja proporción de individuos que se dispersan en ambas especies podría ser debida a la baja disponibilidad de nuevos sitios de reproducción y a los elevados costes que implicarían esta dispersión en términos fisiológicos y de una menor supervivencia (Waser et al. 1994, Plissner & Gowaty 1996). La falta de diferencias significativas entre el carricero común y el carricerín real no es consistente con la hipótesis de que tienen una mayor capacidad de dispersión los migradores de larga distancia frente a las especies sedentarias/migratorias de corta distancia propuesta en algunos estudios (Paradis et al. 1998, Sutherland et al. 2000). La similitud entre los patrones de dispersión de las dos especies se podría explicar por su estrecha relación filogenética, o por las similares presiones evolutivas a las que son sometidas debidas a la común necesidad de cruzar amplias áreas de hábitat inadecuado para llegar a nuevas zonas de cría. Nuestro trabajo, junto con estudios previos, confirma la utilidad de utilizar las grandes bases de datos de anillamiento para estudiar los patrones de dispersión de las aves, algo que hasta ahora se hizo en relativamente pocos casos. Llevar a cabo más estudios similares, sobre más especies y diferentes áreas geográficas, podría permitir una mejor definición y comprensión de los patrones generales de dispersión de las aves explorados por Paradis et al. (1998) y Sutherland et al. (2000). Sin embargo, para obtener información más precisa, en futuros estudios sería oportuno tener en cuenta las diferencias espaciales en el esfuerzo de captura, aunque esto va a implicar disponer de datos detallados sobre la distribución de las aves estudiadas y sobre el esfuerzo de captura en áreas muy amplias (Van Houtan et al. 2010).

Es interesante remarcar la alta consistencia entre los resultados obtenidos en el capítulo 3 y en el capítulo 4. En ambos casos hemos comprobado una buena capacidad de dispersión en las especies estudiadas, y la falta de casos de dispersión entre la isla de Mallorca y la península (capítulo 4) es consistente con el reducido flujo genético encontrado entre estas dos áreas (capítulo 3). Además, los datos de marcaje y recaptura han proporcionado elementos que pueden ayudar a interpretar los resultados de los análisis genéticos, así la falta de diferencias en la distribución de las distancias de recaptura y en filopatría entre la dos especies, sugiere que sean diferencias en otras características (continuidad del rango de distribución, tamaño de población, capacidad de cruzar barreras geográficas) las que determinan la menor diferenciación genética poblacional en el carricero común que en carricerín real (capítulo 3). De hecho, en el carricerín real solo hemos encontrado casos de dispersión entre humedales peninsulares de la costa Mediterránea, la zona donde el rango de distribución de la especie es más continua, donde se concentra una parte importante de la población española (Castany & López 2006), y donde, además, no hay importantes barreras geográficas. Sin embargo, esta comparación entre los resultados de los análisis genéticos y de la información obtenida de los datos de marcaje y recaptura tiene que ser evaluada con prudencia, pues por ejemplo, también la existencia de posibles diferencias interespecificas en los costes de dispersión a nivel de eficacia biológica, podrían influenciar el flujo genético.

La información sobre el carricerín real que hemos obtenido en los capítulos 3 y 4 es completamente nueva, por lo que en futuros estudios sería sin duda interesante ampliar el enfoque y estudiar más poblaciones del suroeste de Europa a través de nuevos datos genéticos y de anillamiento. Esto podría aclarar si hay intercambio de individuos entre la población reproductora del sur de Francia (la más importante en el Mediterráneo occidental con 2000 - 6000 parejas, BirdLife International 2004) y las poblaciones más al este (Italia) y al oeste (España, donde también inverna parte de la población francesa).

Bibliografía

BirdLife International. 2004. *Birds in Europe: population estimates, trends and conservation status.* BirdLife International.

Castany J. 2003. El carricerín real (*Acrocephalus melanopogon*) en el P. N. del Prat de Cabanes-Torreblanca. Doctoral thesis. University of Valencia.

Castany J. & López G. 2006. *El carricerín real en España. I Censo Nacional (2005).* SEO/BirdLife.

Díaz M. 1989. Eficacia de un emético (apomorfina) para el estudio de las dietas de paseriformes granívoros. Ardeola 36: 185-191.

Dyrcz A. 2016. Moustached Warbler (*Acrocephalus melanopogon*). In: del Hoyo J., Elliott A., Sargatal J., Christie D.A. & de Juana E., eds. *Handbook of the Birds of the World Alive.* Lynx Edicions (retrieved from http://www.hbw.com/node/58792 on 29 July 2016).

Dyrcz A., Christie D. A. & Garcia E. F. J. 2016. Eurasian Reed warbler (*Acrocephalus scirpaceus*). In: del Hoyo J., Elliott A., Sargatal J., Christie D.A. & de Juana E., eds. *Handbook of the Birds of the World Alive.* Lynx Edicions (retrieved from http://www.hbw.com/node/58800 on 29 July 2016).

Finlayson M., Hollis T. & Davis, T. 1992. Managing Mediterranean Wetlands and their Birds. The International Waterfowl and Wetlands Research Bureau Spec. Publ. No. 20. The International Waterfowl and Wetlands Research Bureau.

Frankham R., Ballou J. D. & Briscoe D. A. 2010. Introduction to Conservation

Genetics, 2nd ed. Cambridge University Press.

Gainzarain J. A. 2003. Carricero común *Acrocephalus scirpaceus*. In: Martí R. & del Moral J. C., eds. *Atlas de las Aves Reproductoras de España*. DGCN-SEO/BirdLife.

Hansson B., Hasselquist D., Tarka M., Zehtindjiev P. & Bensch S. 2008. Postglacial colonisation patterns and the role of isolation and expansion in driving diversification in a passerine bird. PloS ONE 3: e2794.

Hodges M. F. Jr & Krementz D. G. 1996. Neotropical migratory breeding bird communities in riparian forests of different widths along the Altamaha River, Georgia. Wilson Bulletin 108: 496-506.

Hoi H., Eichler T. & Dittami J. 1991. Territorial spacing and interspecific competition in three species of reed warblers. Oecologia 87: 443-448.

Ibrahim K. M., Nichols R. A. & Hewitt G. M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77: 282-291.

Kennerley P. & Pearson D. 2010. *Reed and Bush Warblers*. Christopher Helm Publishers Ltd.

Koenig W. D., Van Vuren D. & Hooge P. N. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends in Ecology & Evolution 11: 514 – 517.

Kralj J., Procházka P., Fainová D., Patzenhauerová H. & Tutiš V. 2010. Intraspecific variation in the wing shape and genetic differentiation of reed warblers *Acrocephalus scirpaceus* in Croatia. Acta Ornithologica 45: 51-58.

Machtans C. S., Villard M. A. & Hannon S. J. 1996. Use of riparian buffer strips as movement corridors by forest birds. Conservation Biology 10: 1366-1379.

Nathan R., Perry G., Cronin J. T., Strand A. E. & Cain M. L. 2003. Methods for estimating long-distance dispersal. Oikos 103: 261-273.

Neto J. M., Arroyo J. L., Bargain B., Monrós J. S., Mátrai N., Procházka P. & Zehtindjiev P. 2012. Phylogeography of a Habitat Specialist with High Dispersal Capability: The Savi's Warbler Locustella luscinioides. PloS ONE 7: e38497.

Paradis E., Baillie S. R., Sutherland W.J. & Gregory R.D. 1998. Patterns of natal and breeding dispersal in birds. Journal of Animal Ecology 67: 518-536.

Paradis E., Baillie S. R. & Sutherland W. J. 2002. Modeling large-scale dispersal distances. Ecological Modelling 151: 279-292.

Plissner J. H. & Gowaty P. A. 1996. Patterns of natal dispersal, turnover, and dispersal costs in eastern bluebirds. Animal Behaviour 51: 1307-1322.

Poulin B., Lefebvre G. & Mauchamp A. 2002. Habitat requirements of passerines and reedbed management in southern France. Biological Conservation 107: 315-325.

Procházka P., Stokke B. G., Jensen H., Fainová D., Bellinvia E., Fossøy F., Vikan J. R., Bryja J. & Soler M. 2011. Low genetic differentiation among reed warbler *Acrocephalus scirpaceus* populations across Europe. Journal of Avian Biology 42: 103-113.

Schmidt M. H., Lefebvre G., Poulin G. & Tscharntke T. 2005. Reed cutting affects arthropods communities, potentially reducing food for passerine birds. Biological Conservation 121: 157-166.

Silva J. P., Phillips L., Jones W., Eldridge J. & O'Hara E. 2007. Life and Europe's wetlands, restoring a vital ecosystem. Office for Official Publications of the European Communities.

Sutherland G. D., Harestad A. S., Price K., Lertzman K. P. 2000. Scaling of natal dispersal distances in terrestrial birds and mammals. Conservation Ecology 4:16 URL http://www.consecol.org/vol4/iss1/art16.

Valera F., Gutiérrez J. E. & Barrios R. 1997. Effectiveness, biases and mortality in the use of apomorphine for determining the diet of granivorous passerines. Condor 99: 765-772.

Van Houtan K. S., Pimm S. L., Halley J. M., Bierregaard R. O. Jr & Lovejoy T. E. 2007. Dispersal of Amazonian birds in continuous and fragmented forest. Ecology Letters 10: 219-229.

Van Houtan K. S., Bass O. L. Jr, Lockwood J. & Pimm S. L. 2010. Importance of estimating dispersal for endangered bird management. Conservation Letters 3: 260-266.

Van Noordwijk A. J., McCleery R. H. & Perrins C. M. 1995. Selection for the timing of great tit (*Parus major*) breeding in relation to caterpillar growth and temperature. Journal of Animal Ecology 64: 451-458.

Van Vessem J., Hecker N. & Tucker G. M. 1997. Inland wetlands. In: Tucker, G. M. and Evans, M. I., eds. *Habitats for birds in Europe: A conservation strategy for the wider environment.* BirdLife Conservation Series 6. BirdLife International.

Waser P.M., Creel S.R. & Lucas J. R. 1994. Death and disappearance: estimating mortality risk associated with philopatry and dispersal. Behavioral Ecology 5: 135-141.

Winkler D. W., Wrege P. H., Allen P. E., Kast T. L., Senesac P., Wasson M. F. & Sullivan P. J. 2005. The natal dispersal of tree swallows in a continuous mainland environment. Journal of Animal Ecology 74:1080-1090.

Young A. G. & Clarke M. 2000. *Genetics, Demography and Viability of Fragmented Populations.* Cambridge University Press.

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