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Variation in the expression of migratory activity in blackcap (*Sylvia atricapilla*); effects of the origin, environmental conditions, dominance and personalities

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conditions, dominance and personalities”**

"Variación en la expresión de la actividad migratoria en la curruca capirotada (*Sylvia atricapilla*); efectos de origen, condición ambiental, dominancia y personalidad"

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Memoria presentada por la Licenciada Mateja Bulaic para optar al grado de Doctor en Ciencias Biológicas, dirigida por el Dr. Francisco Pulido Delgado del Departamento de de Zoología y Antropología Física de la Universidad Complutense de Madrid.

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1 Summary

“Variation in the expression of migratory activity in blackcap (*Sylvia atricapilla*); effects of the origin, environmental conditions, dominance and personalities”





1.1 Introduction/ Theoretical framework

Migration is a response to predictable seasonal changes through adaptive movements between suitable breeding and wintering sites, allowing the use of their temporarily available resources. It implies substantial energy costs, predation risks and unpredictable weather conditions and food availability along the migratory route, making migration one of the most challenging activities migratory species have to perform. However, the benefits of migration, from seasonal exploitation of resources at both their breeding and wintering grounds, to the elimination of inter- and intraspecific competition in overpopulated habitats, do outweigh its costs. Migration is genetically programmed, being relatively constant in its timing, distance and direction, but in some populations the environment plays a more important role in its expression. In these populations, migratory behaviour is sufficiently plastic to follow variable environmental conditions and adapt by changing to partial or facultative migratory strategy, or even crossing to sedentary way of life, when environmental conditions are favourable. In order to clarify the mechanism which determines if a bird is migratory or resident, the “threshold model of migration” has been proposed describing inheritance and evolution of migratory behaviour. The model assumes that there is a continuous variable underlying migratory activity (e.g. amounts of proteins or hormones) involved in its expression. The threshold on that variable divides the continuous distribution into dichotomous categories defining the phenotype of the individual. Individuals with no migratory activity are classified as residents with their variable value below the threshold, and individuals with values above the threshold are classified as migrants. However, as changes in migratory activity not only depend on the genetically determined position of the threshold but also on environmental variables, the environmental factor was added to the model. This environmental threshold model predicts that individuals at the extremes of the distribution of the variable underlying migration are not affected by environmental factors, while those closer to the threshold can easily be tipped over to one side or the other.

Environments differ in the extent in which environmental conditions vary over time. Thus, certain types of behaviour, like personalities, could be advantageous in some populations but disadvantageous in another, as individuals with different personality types have different abilities, or predispositions, to respond to environmental changes. Little is known, however, whether different life styles, such as migratory or sedentary, can have an influence on personality traits and, if so, how exactly they affect them as during their lives migratory and sedentary populations are faced with different challenges which require appropriate responses.

In addition to genetic differences in migratory behaviour and facultative responses to changing environmental conditions, social conditions may be one of the factors correlated with migration strategies. It is presumed that the cause could be found within social dominance: under conditions when food is scarce, food sources are occupied by dominants, where subordinate individuals, as poor competitors, are the ones most likely to accept the costs of migration in order to avoid competition with dominant individuals. That proved to be true on population level within Iberian Mediterranean area, where resident populations found to be dominant over migratory during the non-breeding season.



1.2 Objectives and results

The aim of this study was to identify and assess the importance of environmental factors for determining migratory behaviour, as well as the effects of different behaviour types and dominance on migratory activity. We therefore investigated migratory behaviour of three Iberian blackcap (*Sylvia atricapilla*) populations differing in their migratory propensity. These populations were selected in order to cover the three main distributions of migratory activity within the “threshold model” (migratory, partially migratory and sedentary populations). The effects of population on personality traits and social dominance were studied with the aim of assessing the importance of these factors for determining differences in migratory behaviour. The combination of measurements of personality traits, dominance and migratory behaviour in the same individuals makes it a powerful integrative approach to achieve this aims.

The study consisted of five parts, in each of these parts a different behavioural component was investigated. In the first part we studied among-population differences in the onset and amount of migratory activity in captivity during autumn migration. The recording of migratory activity in captivity did not demonstrate any differences in the amount of the migratory activity, however, the migratory population showed slightly earlier onset of the migratory activity.

In the second part, we compared autumn migratory activity within and among two types of environment: cages, representing an artificial environment and outdoor aviaries, which represent a more natural environment. We found that keeping birds in aviaries had only a small effect on the expression of migratory behaviour: in aviaries Iberian blackcaps started migratory activity significantly earlier than in cages, there was no effect on the amount of migratory activity. Yet, sedentary population tended to be more active in aviaries than in cages. In these experiments, we also tested for the possible influence of environmental variables, such as temperature and weather conditions, on migratory restlessness. We found that migratory activity of individuals kept in aviaries was affected by weather conditions, as bad weather caused a reduction of migratory activity.

In the third part, we measured “personality” traits for each individual of the three test populations by determining latencies within each experimental setup. The aim was to explore possible population effect on correlations between personality type and migratory propensity. We expected among-population differences, because certain “personality” traits are presumed to be more advantageous for the migratory way of life, while a different set of traits are expected to be favourable in residents. Our results suggest that sedentary populations are more successful in coping with moderate stress than birds from migratory populations, which have longer latencies. Birds from the sedentary population were also the least flexible as revealed in an experiment where the location of food was changed. They held to the usual feeding habits and did not, or only slowly, adapted to the new conditions given by the altered experimental setup. We cannot say much about among-population differences in exploration rate, as analyses did not result in statistically significant



differences between populations. However, results from the memory test suggest that sedentary population tends to have shorter exploring/neophobic latencies.

In the fourth part, we investigated the relation between migratory habits and the capacity for long-term memory by repeating behavioural experiments three times, both in autumn and in the following spring. The observed shortening or maintenance of latencies from the end of the autumn season to the first test in spring indicates that the behaviour learned in the course of the three tests in autumn was maintained at least until spring, i.e. over a period of four months. We have not found any population differences in the learned behaviour.

In the fifth chapter, we determined the relation between migratory propensity and dominance status. We experimentally observed the establishment of social hierarchy between individuals from different populations, based on pairwise encounters. We expected individuals from the sedentary population to be dominant over individuals from the migratory populations. However, migratory individuals showed a tendency towards higher number of initiated interactions which were positively correlated with dominance score. Partially migratory population tended to be in a subordinate position to the sedentary population. In addition, we tested for the correlation between dominance status and personality types finding that individuals with higher dominance score had shorter latencies when approaching the unfamiliar food plate. Moreover, dominant individuals tended to stick to old habits more strongly, which became apparent by the frequent returning to the usual empty feeder when the new one is present. These results suggest that dominant individuals are fast explorers, which are characterized by shorter latencies but less flexibility in adjusting their behaviour to changed food location.

Aside from exploration, another factor responsible for the social hierarchy is the individual's ability to cope with stress. Individuals with high dominance status in pairs tended to lose their position in the hierarchy once they found themselves in larger social group. This may be due to poor stress coping and slow recovery after lost interactions.

1.3 Conclusions

We showed that, contrary to previous studies, blackcaps from all populations, including the sedentary and the partially migratory populations, showed migratory restlessness. We hypothesize that due to the relatively narrow geographical origin of our test populations, the Iberian Peninsula, the among-population differences in migratory behaviour in the wild do not represent genetically different migratory strategies, but are induced by environmental conditions. As these populations are closer to the migration threshold, they seem particularly sensitive to environmental conditions. Further support for this environmental-sensitivity hypothesis was provided by among-population differences in the response to environmental variables. We found that birds from the sedentary population showed the largest, and birds from the migratory population the smallest, change in migratory activity when exposed to a different environment (i.e. aviaries).



We believe that the absence of population differences is a result of housing of all three populations at the specific locality with environmental conditions experienced by migratory populations (Madrid), where the birds were responding to its particular conditions. As a consequence, the migration threshold could have shifted to the left, being the main cause for the expression of migratory behaviour within all three populations.

The initial hypothesis that resident birds are more explorative was not confirmed as we found that birds from the sedentary population are more rigid in adapting feeding behaviour to changes in food location. Moreover, migratory and partially migratory populations showed to be more flexible in their behaviour, discarding faster the empty, but familiar, food source and directing their attention to the new one. While migrating, foraging on stopover sites is time constricted, thus a more flexible feeding behaviour could be advantageous. In addition, we found that the behaviour learned in the course of the tests was maintained over a period of four months, indicating the existence of the long-term memory.

The finding that birds from the migratory population tended to have higher number of individuals marked as dominant over birds from resident or partially migratory populations, may lead towards the assumption of its higher dominance score, and suggest that dominance does not determine migratory behaviour. Difference in dominance between migrants and resident may be due to migrants fuelling up for the autumn migratory season, or higher motivation by early arrival to breeding grounds and territory establishment. Partially migratory population tends to be in a subordinate position when confronted with sedentary population where the observed effect might be explained by the body size hypothesis, with bigger sedentary individuals' dominance tendency. Individuals with higher dominance score tended to be fast explorers, as well as more rigid and less flexible in adjusting their behaviour to changed food location, relying more on their previous experience.

Aside from exploration, another factor responsible for the social hierarchy is the individual's ability to cope with stress. This became apparent in the change of dominance status when dominant individuals were introduced into larger groups where they became subordinate.

In our study we found that among-population differences in migration are mainly caused by differences in environmental conditions and not, as previously suggested, by genetic differences. The environmental conditions given in our experiment induced migratory behaviour in all three populations, although a number of environmental variables (food, density, competition) that previously had been considered important in the control of migration were conducive to sedentary behaviour. We thus have to conclude that neither food availability, nor density, dominance nor personality types are the main environmental determinants of differences in migratory activity among Iberian blackcap populations. Further experiments need to explore the effects of other factors, like air pressure, magnetic fields and the interaction of these factors with those we investigated in our study.



2 INTRODUCTION





2.1 Migratory activity

Migration is an adaptive movement between two distinct areas, usually one being suitable for breeding and the other for wintering, as a response to predictable seasonal changes (Gauthreaux, 1982; Berthold, 1996; Ramenofsky and Wingfield, 2007) allowing the use of temporarily available resources (Dingle and Drake, 2007). It is one of the best described movement phenomena (Dingle and Drake, 2007) in the animal kingdom (Clark *et al.*, 2004). Although migration is present in numerous taxa, it is best known and studied in birds, where a large variety of migratory strategies have evolved.

Looking at the migratory birds' annual cycle, we could say that it is one of the most challenging activities they have to perform (King and Farner, 1959; Kersten and Piersma, 1987; Klaassen and Biebach, 1994). Migration comes with a price, a price of substantial energy costs and the risk of predation and unpredictable weather or food availability along the migratory route, which together can lead to lower survival (Alerstam, 1991; Berthold, 2001; Sillett and Holmes, 2002; Newton, 2007). However, migrants do benefit from the seasonal exploitation of resources at both their breeding and wintering grounds (Greenberg, 1980; Alerstam and Högstedt, 1982), where migration has influenced the evolution of life histories by adjustment of fecundity and survival rates (Bell, 1996; Young, 1996). Aside from two main factors crucial for its evolution; competition and variation in resources in a seasonal environment, other important factors favouring the evolution of migratory behaviour could be the reduction of inter- and intraspecific competition in overpopulated habitats, as well as the avoidance of predators and parasites (Gauthreaux, 1982; Alerstam *et al.*, 2003).

One proposition on how bird migration may have evolved, the “southern-home hypothesis” (Gauthreaux, 1982; Rappole and Jones, 2002; Jahn *et al.*, 2004), claims that birds that were living at low latitudes, where competition was high, moved to the north to benefit from unused resources to maximize their breeding success. Birds that breed at higher latitudes can take advantage of the food peak in spring, abundant nesting locations and long days for foraging. However, they could stay there only for a short period of time, as food decreases and unfavourable conditions emerge as the winter approaches, then they move south again. An opposite idea, the “northern-home hypothesis” (see Gauthreaux, 1982; Salewski and Bruderer, 2007), suggests that birds changed their wintering grounds due to unfavourable conditions at higher latitude, and moved south for the winter. However, Salewski and Bruderer (2007) suggest a synthesis of both hypotheses, the “dispersal-migration theory”, by explaining the evolution of migration through selection after non-directed events as dispersal and colonization, emphasising the importance of dispersal into seasonal habitats as the first step for migration to be selectively advantageous (Salewski and Bruderer, 2007). The theory requires a non-regular movement to a seasonal environment that provides sufficient resources only during breeding season. This movement initially does not need to be controlled by an inherited time and direction program. Birds would reproduce and migrate after breeding, returning for the next breeding season as the parents' migration program becomes heritable (Salewski and Bruderer, 2007).



This genetically programmed departure from and return to the breeding area is considered as obligate migration, being relatively constant in its timing, distance and direction (Newton, 2012). Yet, in some populations the environment seems to play a more important role. Under variable environmental conditions, migratory behaviour has to be able to follow those changes, whether by significant change or gradual displacements of breeding or wintering areas, e.g. slight northward shifts of European wintering/breeding species (Lehikoinen *et al.*, 2013) or the leap in overwintering of sub-Saharan area species to Mediterranean (Newton, 2008), as well as the establishment of new wintering grounds, like we see in some blackcap (*Sylvia atricapilla*) populations overwintering within Great Britain (Leach, 1981). Different ecological factors may promote different migratory strategies (Chapman *et al.*, 2011), such as in partially migratory populations, i.e. part of the population develops sedentary behaviour or decreases migration distance due to improvement of conditions that made possible overwintering closer or at the same breeding grounds (Leach, 1981; Berthold, 1988; Berthold and Terrill, 1988). Another environment-dependent type of migration is facultative migration, where migration is optional, depending on the current environmental conditions being favourable or not for overwintering (Newton, 2012). Rather than completely different categories of migratory behaviour, we can consider obligate and facultative migrants as different parts of the same axis (Newton, 2012), where in both exists a genetic predisposition for migration, but with different phenotypic plasticity for the change from migratory to sedentary strategy or vice versa (Pulido, 2011). In order to clarify the mechanisms which determine if a bird becomes a migrant or a resident, a genetic “threshold model” has been proposed describing the expression, inheritance and evolution of migratory behaviour (Pulido *et al.*, 1996). The principle of the model is based on an underlying normally distributed continuous variable that could be describing, for example, amounts of proteins or hormones involved in the expression of migratory activity and which is correlated with migratory activity. The threshold on that variable divides it into two categories, defining the migration phenotype of the individual. Laboratory experiments on blackcaps proved the model accurate in describing the incidence of migration (Pulido *et al.*, 1996) in the way that individuals with no migratory activity are classified as residents with its variable value below the threshold, and as residents with values above the threshold.

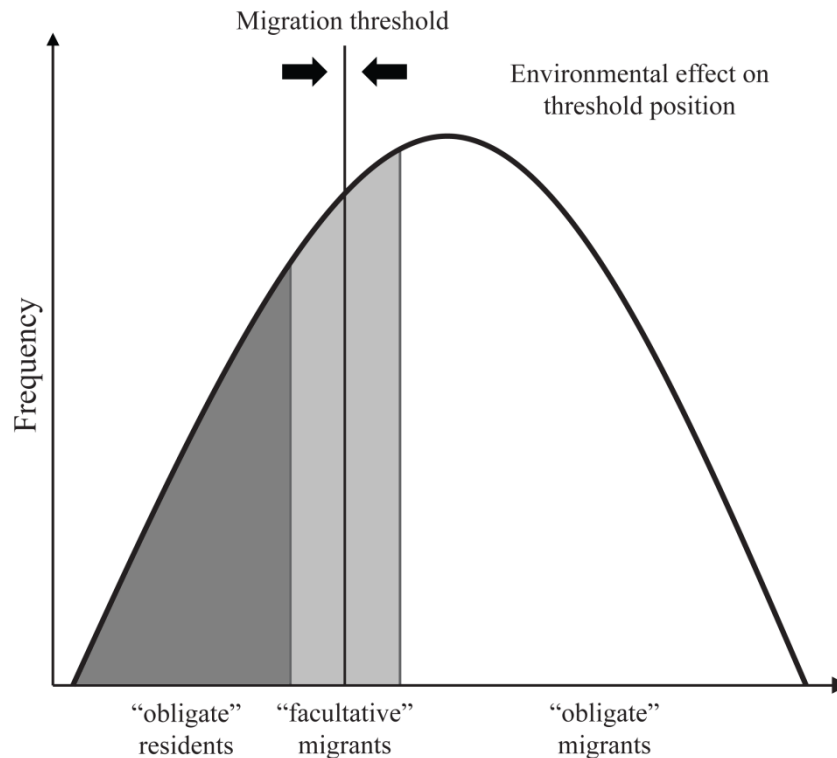


Figure 1: “The environmental threshold model of migration”; describing the distribution of migratory propensity within obligate residents, facultative and obligate migrants taking into account environmental effects on the position of the threshold on the x axis; adapted from Pulido, 2011.

As these experiments on the migration threshold were conducted under artificial conditions, the question arises how the model works and whether it is applicable in natural populations. Therefore, an extension of the “threshold model” was proposed, in which the effects of the environment were considered. This was achieved by including environmental variability, aside from genetic, as one of the determinants of migratory activity (Pulido, 2011). The model predicts that individuals at the extremes of the distribution are not affected by environmental factors, while those close to the threshold can easily be tipped over to one side or the other. Hence, changes in migration activity depend not only on the genetically determined position of the threshold but also on environmental effects. Several environmental factors modifying the propensity to migrate have been identified, such as food availability, temperature and dominance (Pulido, 2011). Yet their contribution in modifying the migration threshold has not yet been assessed, and the importance of other extrinsic (e.g. magnetic field) and intrinsic (e.g. animal personalities) factors is unknown.

In this study, we investigated the effects of personality traits, social dominance and environmental conditions on migratory behaviour with the aim of assessing the importance of these factors for the expression of migratory behaviour. Specifically, we studied three Iberian blackcap populations differing in migratory behaviour. These populations were chosen because they represent three main phenotypes of migratory activity within the “threshold model” (i.e. residency, partial and complete migration) and because we expected that environmental effects are most important in Iberian blackcaps that, if migratory, are short-distance migrants throughout and therefore closer to the migration threshold.



2.2 Environmental influence on migratory restlessness

Many species of migratory birds when held in captivity show an activity that reflects the migratory pattern of the species' wild populations called migratory restlessness or "Zugunruhe" (Berthold, 1973; Gwinner and Czelschlik, 1978; Berthold, 2001). This phenomenon is thought of as the most important evidence supporting the endogenous migration control hypothesis (Berthold 2001). In captivity, migratory birds display a significant increase in activity around the same time the free living populations start their seasonal autumn and spring migration (Berthold, 1973; Berthold, 1990). The observed migratory restlessness corresponded to the activity of naturally occurring migration (Gwinner, 1986b; Berthold, 1990a; Berthold, 1999) by being roughly correlated with the distance, duration and directionality of individuals of the same population in the wild (Berthold, 1996). Common-garden and breeding experiments demonstrated the genetic control of migratory activity and its population specificity (Berthold, 1990; Berthold and Pulido, 1994, reviewed in Pulido and Berthold, 2003). Using hand raised blackcaps, from populations ranging from migratory northern Finland to sedentary Canary Islands and their hybrids, Berthold and Querner (Berthold and Querner, 1981) demonstrated by a common-garden experiment that differences in nocturnal migratory activity were genetic, where migratory-sedentary hybrids displayed intermediate activity values. These experiments also showed that partially migratory populations with selective breeding can become completely sedentary or migratory within few generations (Pulido and Berthold, 2010).

Endogenous (circannual) rhythms have been demonstrated in a number of species (Berthold and Querner, 1981; Gwinner, 1986b; Gwinner, 1990, 2003). First avian circannual rhythms were described on migratory willow warblers (*Phylloscopus trochilus*) that were kept under a constant photoperiod and temperature for three cycles and which kept displaying annual rhythms of moult and migratory activity (Gwinner, 1986a). Later on, they were demonstrated for another 20 species of birds (Gwinner and Dittami, 1990; Holberton and Able, 1992; Berthold *et al.*, 2001; Piersma, 2002). However, the circannual rhythms do not necessarily meet the seasons on the calendar year, they can deviate considerably when birds are kept under constant environmental conditions (Gwinner and Scheuerlein, 1998). Thus, additional synchronizers ("Zeitgeber"), which are present in the natural environment of these birds, are required to adjust the endogenous programs to the occurrence of the biological events within the natural year. It has been found that the most important synchronizer for the migratory events is the photoperiod (Berthold, 1979; Gwinner, 1986b; Berthold, 1988; Helm and Gwinner, 2006). The photoperiod is believed to synchronize the avian life cycle with environmental seasonality, especially in regions out of equatorial area, where it's the most reliable predictor of spring and summer (Dawson *et al.*, 2001). Aside from the migratory activity, it is also responsible for the regulation of circannual rhythms of gonadal size and moult (Gwinner and Scheuerlein, 1998).

It was believed that in captivity migratory activity could hardly be under influence of other environmental factors, aside from photoperiod, that usually affect migratory propensity in the wild, such as food availability, competition, temperature and weather conditions or density (Newton, 2008; Boyle, 2011).



This assumption was disproved by finding that the same factors affecting migratory activity in natural populations may modify migratory behaviour, at least in some captive species, with their intensity varying along the migratory season, between or within populations etc. (see revision in Pulido, 2011).

Most of the attention was focused on studying “Zugunruhe” in migrants, while it was presumed that such phenomenon is not characteristic for residents. Surprisingly, an increasing number of studies comparing the two types of migratory style state that the migratory restlessness might not be unique to migrants. Some resident species express intense nocturnal activity during migratory periods (Smith *et al.*, 1969; Chan, 1994; Berthold, 1996; Helm and Gwinner, 2006; reviewed in Helm, 2006). However, the nocturnal migratory restlessness studied on resident white-crowned sparrows (*Zonotrichia leucophrys*) (Mewaldt *et al.*, 1968), blackcaps (Berthold, 1996), or resident stonechats (*Saxicola rubicola*) (Helm and Gwinner, 2006) was expressed in lower levels than in migratory species. The expression of migratory restlessness within residents was suggested to be a result of an endogenous program, timed by a photoperiodic regime, and displayed when required by environmental conditions (Helm and Gwinner, 2006).

Nocturnal activity doesn't always have to reflect the urge to migrate, as it is hypothesized to be an atavistic trait, expressed at a lower level than within natural populations (Mewaldt *et al.*, 1968; Smith *et al.*, 1969), or assigned to other types of behaviour as juvenile or nocturnal dispersal, nomadism or territoriality (Berthold, 1988; Mukhin *et al.*, 2005; Mukhin *et al.*, 2009). To prove that it was indeed an expression of migratory behaviour, some authors have described the behavioural characteristics of the night activity within some species. In blackcaps it is defined as displays of intense seasonal nocturnal activity within captivity, consisting of hopping, climbing, flying and wing whirring while not leaving the perch or side of the cage, described as “flying with the brakes on” (Berthold, 1988; Mukhin *et al.*, 2005; Mukhin *et al.*, 2009). In resident and migratory white crowned sparrow (Agatsuma and Ramenofsky, 2006; Coverdill *et al.*, 2011, respectively) migratory activity is characterized by beak-up and beak-up flight behaviour and quiescent phase, believed to represent the gathering of information, looking at the night sky, and taking off for migratory flight. However, such studies are scarce on resident species.

In this study, we tested for the presence of “Zugunruhe” in blackcaps held in captivity that can be conveniently measured for this nocturnal migrant. Migratory activity was measured for the individuals both inside cages and aviaries. Berthold's studies on this species showed distinct migratory restlessness, with sample populations ranging from southern Finland to Canary islands (Berthold and Querner, 1981). Here, we wanted to compare populations on a smaller geographical scale, the Iberian Peninsula, within which we would look for differences in migratory restlessness among three different populations: a migratory population from the north (Madrid), a partially migratory population from the central Mediterranean coast (Cocentaina) and a sedentary population from the very south of the Iberian Peninsula (Tarifa) (Tellería *et al.*, 2001). As the migratory activity was usually measured inside individual cages, we wanted to quantify and describe migratory behaviour under more natural conditions. We therefore kept and measured migratory activity in



aviaries, which could be considered as a semi-natural environment. This has, to our knowledge, not been done before. By conducting experiments under these conditions, we hope to obtain more valid results, allowing us to determine the environmental migration threshold model in a less artificial environment.

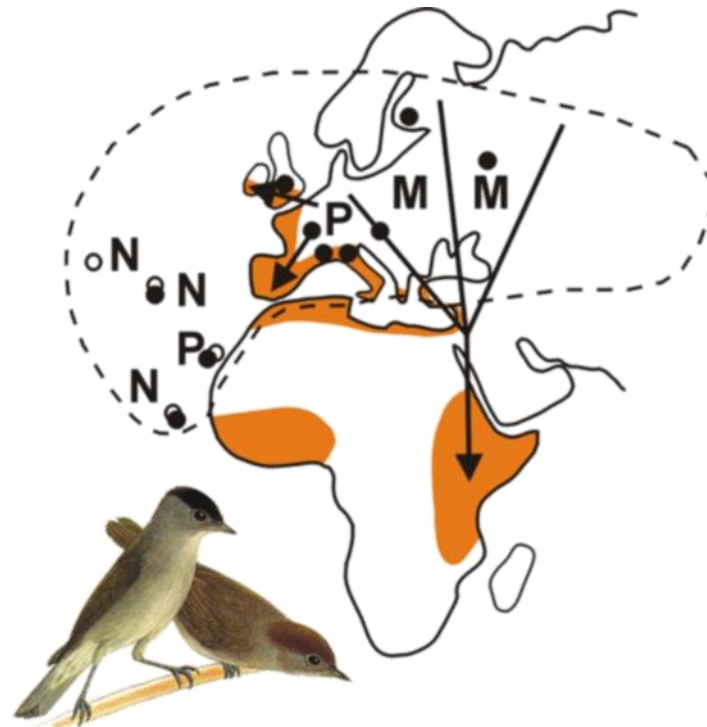


Figure 2: Distribution of the three migratory strategies within the blackcap; M-migratory populations, P-partially migratory, S-sedentary populations. Broken line marking the breeding area, orange overwintering area. Arrows are representing the main migratory routes to the main overwintering sites.

2.3 Personality traits and their correlation with migratory activity

Reactions of individuals to new and uncertain situations differ in a range of species, but at the same time, these behavioural responses are displayed with consistency both within individual and between individuals (Sih *et al.*, 2004a; Sih *et al.*, 2004b). Such consistent differences in behaviour have been termed “animal personality” (Gosling, 2001; Réale *et al.*, 2007) or “behavioural syndromes” (Sih *et al.*, 2004a; Sih *et al.*, 2004b) if they describe suits of correlated behavioural traits. They reflect differences in how an animal copes with new information by developing different behavioural (Sih *et al.*, 2004b; Carere *et al.*, 2005; Bell *et al.*, 2009) and physiological strategies (Carere *et al.*, 2001; Carere *et al.*, 2003; Carere and van Oers, 2004).

Different environments tend to favour different behaviours, so a certain personality could be preferred in one environment and result to be a huge disadvantage for an individual or the population in another. As well, different personality types have different abilities, or predispositions, in adjusting and responding to changes within environments (Koolhaas *et al.*, 1999; Coppens *et al.*, 2010; Mathot *et al.*, 2012). This flexibility is presumed to be a consequence of environmental stimuli, to a certain extent, on the behaviour of an individual (Coppens *et al.*, 2010; Koolhaas *et al.*, 2010), where flexible individuals are able to respond



optimally to presented stimuli. Faced with a new and unfamiliar environmental situation, some individuals gather detailed information and rely on them, changing their behaviour accordingly. These reactive individuals, or “slow explorers”, pay close attention to their environment coping with the challenges that it brings (Sih *et al.*, 2004b; Niemela *et al.*, 2013). On the other hand, proactive individuals or “fast explorers” explore the environment more quickly, but usually are rigid, less innovative and prone to routine-like behaviour in their response (Verbeek *et al.*, 1994; Verbeek *et al.*, 1996). That is why proactive individuals have an advantage over reactive ones in a relatively constant environment, where conditions do not change or in highly variable environments, where flexibility is not advantageous (Sih *et al.*, 2004a; Niemela *et al.*, 2013). However, it takes them longer to adjust to moderately variable environments where reactive individuals are better adapted (Carere, 2003b; van Overveld and Matthysen, 2013). Hence, certain personalities will be favoured by selection depending on the distinct environmental conditions that the population of concern is experiencing at a given moment, and having higher variation in behavioural traits could be beneficial for species enabling them to perform better under an ample range of conditions (Sih *et al.*, 2012). Hence, the presence of variation in animals personalities could be important in ecological and evolutionary processes (Wolf and Weissing, 2012).

Little is known whether different life styles, such as migratory or sedentary, have an influence on personality traits and, if so, how exactly they affect them (Mettke-Hofmann *et al.*, 2005b). There are indications based on comparative studies that migratory and sedentary species differ in exploratory and neophobic behaviour (Greenberg, 1983; Mettke-Hofmann *et al.*, 2002; Mettke-Hofmann *et al.*, 2005b). For that reason, it would be interesting to compare personality types with migratory behaviour of different populations or species (Réale *et al.*, 2007), as during their lives, migrants and residents are faced with different challenges which require appropriate responses. Migrants are confronted with unfamiliar habitats on their migratory routes and stay in a particular area for a relatively short period of time, while residents remain in the same area and have to cope with seasonal changes. Thus, residents would particularly benefit from an extensive knowledge of their environment (Mettke-Hofmann *et al.*, 2005b). Studies on 10 parrot species (Mettke-Hofmann *et al.*, 2002) showed that resident species approached novel object sooner than the migrants. Also, when neophobic behaviour was tested in resident Sardinian warblers and migratory garden warblers, Sardinian warblers were less neophobic and more explorative (Mettke-Hofmann *et al.*, 2005a). Within the third type of migratory strategy, partial migration, individuals could change their strategy – from being migratory to becoming resident or vice versa (Schwabl, 1983; Heldbjerg and Karlsson, 1997; Able and Belthoff, 1998). For that reason, we should expect partial migrants to have the capacity to easily adjust to both conditions favouring migration and conditions favouring residency, rendering themselves an interesting model for studying the environmental control of migratory behaviour.

Most of the studies in passerine species have obtained similar results regarding migration propensity and type of “personality”. But, there are some opposite examples in the literature, like for the blue tit (*Cyanistes caeruleus*). Nilsson and his colleagues (Nilsson *et al.*, 2010) tested a migratory and a sedentary



population in a novel object experiment, and found that migratory individuals had shorter latencies in approaching the novel object than the residents. One of the possible reasons for this kind of results is that this species may not be suitable for this kind of studies, as the blue tit belongs to a cluster of mainly sedentary species with slow migration speed and distances, which only occasionally migrates (Nilsson *et al.*, 2008). For this reason, more studies are needed before we can generalize the relation between personality and migratory behaviour and assess whether this relation varies among species.

2.4 Dominance status and its correlation to migratory activity

In gregarious species, individuals interact with each other. The emerging social relationships are responsible for the formation of dominance hierarchies within the group, where each individual can be characterized by its social rank (Chase, 1980). Dominance status is related to fitness; it can affect both survival and reproductive success (Fox *et al.*, 2009). Dominant individuals may benefit from priority access to food resources (Hogstad, 1989) and foraging sites that are safe from predators (Ekman, 1989), or are more attractive for the opposite sex, resulting in greater mating success of the territory holder (Otter and Ratcliffe, 1996). Thus, group members might differ in motivation (e.g. for foraging), which could further be reflected in the individual's behaviour (Fox *et al.*, 2009). For example, subordinate individuals may be forced to look for other resources if dominant individuals block the access to the familiar ones. This will increase the potential costs and risks of exploration (Laland and Reader, 1999). An example can be found in jackdaws (*Corvus monedula*), where lower ranking individuals are faster in detecting and exploring novel locations (Katzir, 1982). The social context is responsible for this rank dependent behaviour, forcing low ranking individuals to be faster explorers and less neophobic in search for food. Once individuals are moved to another group, we cannot predict their behaviour with certainty (Fox *et al.*, 2009).

Usually, some age-sex classes are considered as subordinate, e.g. females and juveniles are usually dominated by adults and males throughout the non-breeding season, and generally, they migrate further than the dominants (Terrill, 1987). The hypothesis derived from these findings is that the cause for differential migration could be found within social dominance (Gauthreaux, 1978). “The dominance hypothesis” states that when food is scarce and food sources are occupied by dominants, subordinate individuals, as poor competitors, are the ones most likely to accept the costs of migration in order to avoid competition with dominant individuals (Gauthreaux, 1978; Ketterson and Nolan Jr, 1979). This hypothesis has received support from several studies. Lundberg, for instance, found that juvenile and female European blackbirds (*Turdus merula*) lost their body fat and weight in mid-winter, while, at the same time, males and adults reached their peak body mass (Lundberg, 1985). The results suggest that the majority of migrating individuals of the studied population in autumn migratory season is formed by females and juveniles. For example, Terrill (1987) found that in dark-eyed juncos (*Junco hyemalis*) restriction of food increased “Zugunruhe” in both dominant and subordinate individuals, yet, the increase was much higher in subordinates. Subordinates also continued displaying migratory activity during the winter period after dominants and controls had stopped



“Zugunruhe”. Later in autumn migration, when the food was again given *ad libitum*, migratory activity decreased significantly in all individuals. These results indicate that towards the end of autumn migration, migratory behaviour is subject to ecological and social conditions that influence the probability of survival during the winter (Terrill, 1987; Newton, 2012).

However, some studies have rejected this hypothesis. Rogers (Rogers *et al.*, 1989) studied two populations of dark eyed juncos (*Junco hyemalis*), that were crossing different distances within autumn migration. He found that individuals with shorter migration distances were dominant over individuals with longer migration only in half of the pairwise encounters. Additionally, juveniles wintering at northern latitudes were subordinate to more dominant old males wintering further south. Therefore, presumably, dominance status and migratory activity could be correlated within individuals or between populations.





3 GENERAL AIMS





The central aim of this thesis is to identify and assess the importance of environmental factors for determining migratory behaviour. For this I explored the effects of intrinsic (exploratory behaviour, dominance) and environmental factors on migratory activity. In my thesis I approach the following questions:

- (1) Can we measure differences in the onset and amount of migratory activity in a common-garden experiment in captivity between three populations differing in migratory behaviour in the wild? (Chapter 1)
- (2) Do individuals from these populations differ in their nocturnal activity patterns? Is there a measurable difference in night activity patterns between populations? (Chapter 2)
- (3) Are there any differences in the migratory activity between two environments; between activity inside cages and aviaries as the more “natural” environment? Is behavioural expression of migratory restlessness different within aviaries compared to cages? (Chapter 2)
- (4) Is there an influence of environmental variables, specifically temperature and weather conditions, on migratory activity? (Chapter 2)
- (5) Is it possible to identify and describe the "personalities" in the blackcap (*Sylvia atricapilla*), from 3 Iberian populations differing in migratory strategy, and determine the behavioural elements which compose them, especially items related to exploratory behaviour? (Chapter 3)
- (6) Based on the set of novelty experiments, is it possible to explore inter-population differences and see if a generalization of population's personality types could be applied depending on their migratory strategy? (Chapter 3)
- (7) Is there a long term memory in our subject species? Does the memory of information learned during the autumn migratory season persist until the spring migration period and can this information be used in the corresponding situations? (Chapter 4)
- (8) Is it possible to observe the establishment of social hierarchy within pairs of individuals belonging to different populations, based on their pairwise encounters? Is there a correlation between dominance status and migratory activity? Does the position of an individual within a social hierarchy determine whether or not this individual will migrate? (Chapter 5)
- (9) Is there a correlation between dominance status and personality type? Are dominant individuals predominantly fast explorers and subordinate individuals slow explorers? (Chapter 5)
- (10) Does the dominance rank of an individual obtained by interactions with a conspecific change once that individual finds itself in a larger social group? Do dominant individuals retain their superior position or do they become subordinates? (Chapter 5)





4 GENERAL METHODS





4.1 Blackcap (*Sylvia atricapilla*), the model species

The blackcap is a common and widespread warbler, and large number of studies on migratory activity performed on the species makes it an ideal model for studying mechanisms correlating migratory styles, personality types and dominance. It is one of the most abundant passerines in the western Palaearctic (Shirihai *et al.*, 2001), obviously named by the colour of the male's crown or cap, extending to the eye level, while female's cap is brown-reddish in colour. The blackcap is a nocturnal migrant, which shows different migratory strategies across its range: long distance migrants, mostly in northern and eastern Europe, partial migrants in southern Europe, and sedentary populations in southern Iberia and the and Macaronesia (Berthold, 1996; Shirihai *et al.*, 2001; Tellería *et al.*, 2001) (Figure 3). When held in captivity, this species displays nocturnal migratory restlessness during the autumn and spring migratory seasons, which can be easily recorded under laboratory conditions (Berthold *et al.*, 1972). The proximate causes underlying different migratory strategies have been studied in “common garden” experiment, a powerful method for detecting genetic differences. Using this approach it was demonstrated that within- and among-population differences in migratory behaviour have a genetic basis (Berthold and Querner, 1981; Berthold *et al.*, 1990; Pulido *et al.*, 2001; van Noordwijk *et al.*, 2006). The “common garden” experimental setup is ideal for studying whether personality types and dominance ranks are correlated with genetically differences in migratory behaviour.

Most of comparative studies on migratory behaviour of the family “Sylviidae” have been done on continental scale, populations ranging from the north to the south of the Europe. In contrast, this research studies geographic differences in migratory behaviour on a smaller geographical scale - the Iberian Peninsula, where blackcap populations have been well studied for their migratory behaviour in the wild (Tellería and Carbonell, 1999, Pérez-Tris *et al.*, 1999; Perez-Tris *et al.*, 2004; Tellería *et al.*, 2008; Tellería *et al.*, 2013; Morganti *et al.*, 2015). Ringing data show that in the north and centre of the Peninsula, blackcap populations are migratory, while in the south populations numerous are sedentary (Finlayson, 1981; Cuadrado, 1994; Cantos, 1995; Pérez-Tris *et al.*, 1999). Populations of the Mediterranean Coast, particularly in the north and centre (i.e. in the regions of Catalonia and Valencia), are believed to be partially migratory (Morganti *et al.*, 2015). Apart from the difference in the migratory propensity, there are slight differences in morphology. Migratory populations in the north have more pointed and longer wings, while resident populations have more rounded and shorter wings (Tellería and Carbonell, 1999).

4.2 Collection and maintenance of birds

Over the timespan of three consecutive years 2010 (N=33), 2011 (N=33) and 2012 (N=36), we captured juvenile blackcaps during summer at an age of 2-3 months in three Iberian populations. The rationale for selecting these populations was to study populations with different migratory strategies (Figure 4): Madrid (Pinilla del Valle 40°55'N, 3°49'W) represents a migratory population, Cocentaina (38°44'N, 0°26'W) a



partially migratory and Tarifa (Los Barrios 36°11'N, 5°36'W) a sedentary population (Tellería et al., 2001). All birds were trapped between mid-June and the beginning of August. After capture, each bird was banded with a colour ring that had a unique colour code. This made it possible to easily identify each individual during the study. Birds were then transported to our study facility in a restricted area within the Madrid's natural park Casa de Campo (40°25'N, 3°45'W), and were kept in individual cages (45x23x38cm) with two movable perches, feeder and two drinkers. Alternatively, they were kept in outdoor aviaries (3x2x2.3m), equipped with 6 perches in three corners, two feeders, two water plates, and natural undergrowth and overhanging holm oaks (*Quercus ilex*). In both treatments, food consisting of fresh fruit of the season (pomegranates, apples, figs, and pears), mealworm, industrial food for insectivorous birds with addition of vitamins "Raff, Patée con insetti" and water were given ad libitum, and replenished daily. Birds kept in outdoor aviaries were exposed to the natural photoperiod (initial light:dark hours ratio 13L:11Dh; final 9L:15D) and temperature fluctuations (range: 1°C-40°C in the sun). Individuals housed in indoor cages were exposed to attenuated outdoor temperature conditions, i.e. due to the shelter minimum temperatures were about 4°C warmer and maximum temperatures about 4°C cooler than in the aviaries. Birds indoors received natural daylight entering through two windows (1x1.5m). To reach outdoor light intensity, the room was illuminated with two additional lights (2 compact fluorescent lamps, Megaman WL 130 Compact 2000 HPF, with a power of 30 W, a light intensity of 1620 lumen and a colour temperature of 6500 K=daylight), which were adjusted to the natural photoperiod weekly using the data on sunset and sunrise published by the weather station of Barajas, Madrid (http://www.tutiempo.net/tiempo/Madrid_Barajas/LEMD.htm). As migratory activity is not expressed in complete darkness (Helms, 1963; Berthold 1996) a night light (2-3 Lux) was mounted centrally in the experimental chamber, which was switched on throughout the experiment.

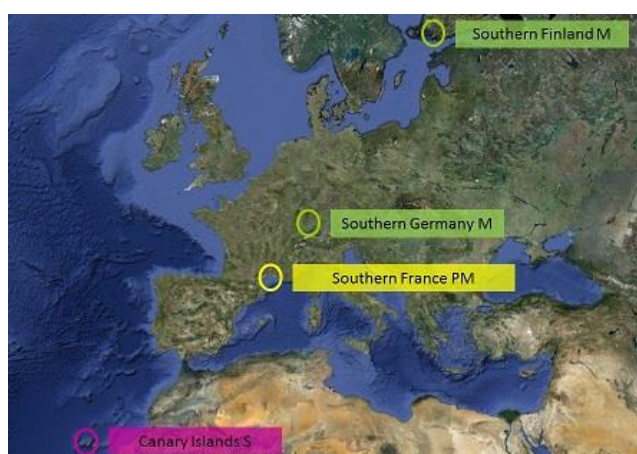


Figure 3: Distribution of the populations used in previous studies ranging from the north of Europe in Finland to the very south in Canary Islands; M-migratory populations, PM-partially migratory, S-sedentary populations



Figure 4: Distribution of the three populations within the Iberian Peninsula used in our study; Madrid–migratory population, Cocentaina–partially migratory population, Tarifa–sedentary population



4.3 Methods of the Chapter 1 and 2

4.3.1 Measuring migratory restlessness

Nocturnal activity, also known as migratory restlessness or “Zugunruhe”, is a good indicator of a birds’ migratory activity in captivity (Berthold, 1992). This nocturnal activity is characterized by wing whirring, intents of flying around the cage and jumps. The activity was recorded from September through May of the following year for all three experimental years. Nocturnal activity was quantified through video revision for the autumn migration period only as the amount of perch contacts per 30min intervals during the lights out period (16:00 h to 10:00 h of the following morning). Activity inside cages was measured as the amount of perch contacts recorded through micro switches placed under two movable perches that were connected to the Microscript® recording system (Berthold *et al.*, 1972).



Figure 5: An image showing the nocturnal restlessness in the blackcap held inside the cage during its autumn migration; the bird could be seen sitting while wing whirring



Figure 6: Screen shot of the infrared night video of the nocturnal restlessness inside the outdoor aviaries; the bird could be seen sitting on the upper right perch

4.3.2 Description of types of behaviour displayed during migratory restlessness

So far, many studies have described migratory behaviour in captivity, but studies on migratory activity in “natural” or “semi-natural” environments, like outdoor aviaries, are lacking. Given it has been criticised that migratory restlessness as measured in a laboratory may not reflect actual migratory behaviour in the wild (Helm, 2006; Rappole, 2013) and that current models of the control of the expression of migratory activity predict that in populations close to the migration threshold the expression of migratory behaviour should differ among environments, we analysed and described migratory restlessness both for birds kept in cages and aviaries. By filming this nocturnal activity, we could test whether it was truly a display of migratory restlessness (Gwinner and Czelschlik, 1978; Berthold and Querner, 1988; Berthold *et al.*, 2000), and not confounded by other behaviours, like escape behaviour or response to disturbances by neighbouring birds displaying migratory activity. Each bird was filmed with a surveillance camera on infrared mode installed for a group of cages and one installed within each aviary. Videos were analysed for the every other night during the 3 months period by the same observer. Night activity was quantified, by watching the first 30min of each



hour for 2min for every 10min period (e.g. 0:00, 0:10, 0:20, 0:30). Observed behaviour was assigned to one of three following categories: 1-hopping on the perch; 2-wing whirring and 3-flying. The dominant behaviour was calculated through the frequency of each type of observed behaviour. Total amount of activity was determined by counting the number of active 2-minute intervals.

4.4 Methods of the Chapter 3 and 4

4.4.1 Measuring “personality”/behavioural traits

In all three experimental years, experiments were conducted both in autumn and spring, during the naturally occurring migration period. These two periods were selected because during their first autumn migration birds would be exposed to unfamiliar environment if they were to migrate, while residents should remain in their year-round, familiar environment. Therefore, we would expect that differences in novelty reactions and environmental assessment to be largest during that period. In addition, it has been shown that object exploration peaks in spring, suggesting that knowledge about the surrounding environment before and during the breeding season is of particular importance (Mettke-Hofmann 2007).

The usual methods for testing personality-related behaviour in birds are novel-environment experiments for testing exploratory behaviour (Veerbek *et al.*, 1994, Dingenmanse *et al* 2002), novel-object experiments for testing neophobia (Nilsson *et al.*, 2010) and learning experiments for testing neophobia and cognitive abilities (Marchetti and Drent 2000; Carere, 2003a). In this study, we used similar tests for determining personality type.

4.4.2 Moderate stress experiment

We tested birds from 3 blackcap populations for possible differences in response to a moderately stressful situation. We were interested in latency, i.e. the amount of time necessary for an individual to feed from the usual feeder and overcome the effect of the stressful situation. The observer, to whose presence the birds were already accustomed to, would remove the usual feeder for some 20-30min to deprive the individuals of food, and following that return the original feeder with newly replenished food.

4.4.3 Exploration test

A new type of feeder would be introduced into the cage, while the usual feeder remained empty and was immediately returned to the cage. This allowed us to control for rigid behaviour, i.e. the “error” committing. The aim of this experiment was to test for differences in the way individuals adjust their feeding behaviour to an “altered” environmental situation. With this experiment we intended to study if and how birds change their feeding routines, and investigate if there are among-population differences in their responses.



4.4.4 Memory test

The design of this experiment was similar to the previous one, except that the new feeder was covered with a piece of white paper of the same dimensions in order to prevent the food to be seen. As the previous similar experiment was repeated 3 times, it was expected that in the course of the exploration test birds would learn the new location of the food. In this experiment the novelty is the hidden food. By hiding food in a previously learned location, birds could use previously established routines in exploration but needed to use their cognitive abilities to apply that experience and learn the conditions within the new situation.



Figure 7: Experimental setup for the exploration test; the empty usual feeder placed at the same time as the new feeder, filled with food, at the cage floor



Figure 8: Experimental setup for the memory test; the newly added feeder at the cage floor now covered with a piece of paper to hide the food.

4.5 Methods of the Chapter 5

4.5.1 Dominance experiments

Dominance experiments were conducted inside aviaries at the end of spring migration season, i.e. in April and May. This period was chosen because more individuals were available for testing once released into aviaries, and because at this time they were still migratory active. We determined dominance ranks by observation and recording of active and passive displacements (there were too few aggressive interactions), where the subordinate birds would repeatedly hand over the perching site to the dominant ones (Pravosudov *et al.*, 2003). Each experiment was conducted three times within a period of 5-6 days.

4.5.2 Dominance tested in pairs

Two individuals were introduced to an unfamiliar aviary and observed for dyadic encounters for about 10 min. This set-up allowed us to assign within-pair dominance ranks. The paired individuals would be of the



same sex, but from different populations (Madrid-Tarifa, Madrid-Cocentaina, Cocentaina-Tarifa), to test for possible differences in dominance between populations. The individual's final dominance rank was determined by analysing dominance interactions. A score of one would be assigned to the bird that would keep the perching site when another individual is approaching; or take over a perching site that it is approaching to, while a score of zero is assigned to the individual that would fail to keep the site when faced with an approaching individual, or fail to overtake it.

In addition to same sex pairs, males and females of the same population were paired up in 9 pairs in winter of 2013 (January), to determine dominance relations between males and females, as the literature usually states males' dominance over females (Rubolini *et al.*, 2004; Dierschke *et al.*, 2005; Arizaga, 2011).

4.5.3 Dominance tested in groups of four

Social groups of four individuals were created by transferring two already existing pairs into one unfamiliar aviary. The groups consisted of individuals of the same sex (i.e. only males or only females) but from different populations. In this experiment, the same types of interactions were observed, and same scoring system was used as in the previous experiment. The aim of this experiment was to observe how an individual with an established social rank within pairs would change dominance rank in the presence of more and unknown individuals. We were particularly interested in studying whether dominant individuals would stay dominant, or if they would become subordinate after losing some of the interactions (see Verbeek, 1999).



Figure 9: Two male blackcaps housed within an aviary



Figure 10: Two female blackcaps housed within an aviary



5 GENERAL RESULTS AND DISCUSSION





5.1 Chapter 1

“How the measurement of migratory restlessness under semi-natural conditions can help us gaining a better understanding of the control of bird migration”

All three populations of blackcaps, which we chose for this study because they show different migration strategies in the wild, displayed similar amounts of nocturnal activity during the autumn migration season. This result was particularly surprising, for the birds from the Tarifa population, which are resident and do not show migratory activity in the wild. Moreover, we did not find among-population differences in the proportion of birds showing migratory activity. Actually, only one bird, from the partially migratory population from Cocentaina, did not show migratory activity. In contrast to these results, we observed differences in the onset of migratory activity. Birds from the Madrid population started autumn migratory activity earlier than birds from the other two populations. There are two possible explanations for this difference in migration onset: firstly, it could reflect migratory phenology of the free living populations, as the more northern migrants should leave earlier in order to cover longer distance to the wintering grounds. Secondly, among-population differences in hatching date of our experimental birds could have been the cause as there is a strong positive correlation between hatching date and onset of autumn migratory activity (Pulido *et al.*, 2001a; Pulido *et al.*, 2001b; Coppack *et al.*, 2001). We do not know the exact hatching date as our birds were caught as juveniles. However, birds from the Madrid population also demonstrated a significantly longer duration and later end of migratory activity. Together with the earlier onset of migration, these results are characteristic of more migratory bird populations. We, therefore, believe that it is unlikely that observed differences are only due to the possible among-population variation in hatching date.

Expression of migratory activity within resident populations has been previously documented (Smith *et al.*, 1969; Chan, 1994; Berthold, 1996; reviewed in Helm and Gwinner, 2006). However, it was expressed in significantly lower levels than in migratory species or populations (Mewaldt *et al.*, 1968; Berthold, 1996; Helm and Gwinner, 2006). Although variation in the expression of migratory activity has a strong genetic component (Berthold, 1996; Pulido and Berthold, 2003; Pulido, 2007), the environment plays an important role in its inhibition or release (van Noordwijk *et al.*, 2006; Helm 2006; Pulido, 2011).

According to the “environmental threshold model” of migration, environmental variables might change the expression of migratory activity for the individuals close to the migration threshold, determining whether an individual is a migrant or a resident (Berthold, 1984; Lundberg, 1988; Adriaensen *et al.*, 1990; Pulido, 2011). Individuals with extreme values of the migration propensity variable, such as long-distance migrants or resident populations, will be further away from the threshold and largely insensitive of the influence of the environmental variation on their migratory phenotype (Pulido, 2011); being “environmentally canalized” (Pulido and Widmer, 2005). It is presumed that resident populations closer to the threshold may have cryptic variation in migration propensity. As a consequence resident populations may rapidly adapt to changes in



environmental by expression of the migratory phenotype (Pulido, 2011). We hypothesize that within the Iberian Peninsula among-populations differences in migratory behaviour are primarily due to differences in environmental conditions rather than being genetic. In this region, both resident and migratory populations could find themselves close to the migration threshold, therefore being particularly sensitive to environmental conditions. By housing birds from all three populations in a locality with environmental conditions experienced by migratory populations (Madrid) (Tellería, 2001) may have shifted the migration threshold to the left. As a consequence, migratory behaviour was expressed in birds from all three populations, including the population considered as resident. Small differences in latitude, photoperiod, humidity atmospheric pressure or temperature, compared to Tarifa and Cocentaina, might have been sufficient for changing the expression of migratory activity of the partially migratory and resident population. Having in mind the species' general geographic variation in migratory behaviour and the possibility of its rapid adjustments to climate changes (e.g. the establishment of a new wintering area, Berthold and Terrill, 1988), we may consider the observed change in migratory activity as a flexible response of the partially migratory and resident populations to poor wintering conditions at the Madrid location. In order to confirm this crucial environmental influence on the population phenology, it would be interesting to house birds of these three populations in Tarifa, under the environmental conditions that do not induce migratory activity in the wild. Under these conditions we would expect Tarifa birds not to display “Zugunruhe” and birds from Cocentaina and Madrid to repress their migratory activity.



5.2 Chapter 2

“Migratory behaviour in Iberian Blackcaps (*Sylvia atricapilla*) supports the environmental threshold model of migration”

The main aim of this thesis was to study the potential effects of environmental variables on migratory activity (“environmental threshold model of migration”, Pulido, 2011). For this reason, we housed individuals from different populations in one of two distinct conditions, cages and aviaries; and investigating the reliability of migratory activity data obtained in aviaries, a semi natural environment that allows birds to fly in captivity. We found that keeping birds in aviaries had only small effects on the expression of their migratory behaviour. However, Iberian blackcaps started migratory activity significantly earlier in aviaries than in cages. The mean amount of migratory activity, however, did not generally differ. However, birds from Tarifa tended to be more active in aviaries than in cages. In addition, we found that the migratory activity of individuals kept in aviaries was affected by weather conditions. Meteorological variables, that have been used to characterize “good” or “bad” weather, have been previously identified as one of the most critical proximal cues determining the onset of migratory activity (e.g. barometric pressure, Bagg *et al.*, 1950; temperature, Lack, 1960; direction of winds, Richardson, 1978; cloud cover, Alerstam, 1978). Generally, “bad” weather caused a reduction of migratory activity.

The “environmental threshold model” predicts that partial migrants, sedentary and migratory populations close to the migration threshold should show more plasticity in the expression of migratory behaviour and a stronger response to specific temperature and weather conditions than more migratory populations. In more migratory populations migratory behaviour should vary little among different environments (e.g. cages, aviaries or the wild), as these populations find themselves away from the migration threshold. Therefore, the expression of migratory behaviour is supposed to be more canalized (Pulido, 2011). In accordance with these predictions, our blackcaps from the sedentary Tarifa population demonstrated higher plasticity by a tendency of being more active in aviaries than in cages, presumably due to environmental factors that shifted the migration threshold towards. In contrast to this result, we found no difference in migratory activity between birds from Cocentaina held in cages and aviaries, probably due to the small sample size (caged birds N=3, aviaries N=4). We also found no differences in migratory activity between the two experimental conditions for birds from the Madrid population, which showed the smallest differences of the three populations.

In our experiments birds from all three populations displayed migratory activity in aviaries. The fact that in our experiment in aviaries (the amount of) migratory behaviour did not change, compared to cages, may indicate that by keeping birds in aviaries we did not modify the crucial environmental factors decisive in determining whether a bird close to the migration threshold will migrate or not. We hypothesize that these factors, which were identical in cages and aviaries, are those we couldn’t control and which may be associated



with geographical location (e.g. photoperiod, magnetic field, etc.) or with air pressure. Apparently, the shift of the threshold induced by keeping birds in aviaries was not sufficient to observe different migratory strategies between populations, yet it did affect birds from the Tarifa population, which seem to be more sensitive to environmental changes, confirming the predictions of the “environmental threshold model”.

Aside from quantifying migratory activity inside cages and aviaries, we described the predominant types of behaviour and correlated them with the previously described characteristic migratory behaviour in captivity (wing whirring, “beak-up flight”) (Gwinner and Czelschlik, 1978; Berthold and Querner, 1988; Berthold *et al.*, 2000; Agatsuma and Ramenofsky, 2006; Coverdill *et al.*, 2011) in order to test whether the observed locomotory activity was true migratory activity. Alternatively, this nocturnal activity could be atavistic, non-functional behaviour observed in resident birds (Mewaldt *et al.*, 1968; Smith *et al.*, 1969), or associated to other types of behaviour, as juvenile or nocturnal dispersal, nomadism or territory acquisition and maintenance (Berthold, 1988; Mukhin *et al.*, 2005; Mukhin *et al.*, 2009).

We show that birds inside cages were predominantly displaying wing whirring, previously described as “wandering by wing whirring in a sitting position” or “flying with the brakes on” (Berthold and Querner, 1988; Berthold, 2000), and that in aviaries flying was the predominant behaviour in all three populations, which was previously undescribed. This difference in type is probably only a consequence of different housing conditions as the caged birds are confined to a much smaller place limiting their movement, making it impossible for them to display their “natural” flying behaviour during migration. We confirm that the recorded nocturnal activity in cages (i.e. predominantly wing whirring) was indeed a display of migratory behaviour (i.e. nocturnal flight), not just escape behaviour or a locomotory activity due to possible disturbances. We claim that it corresponds to the flying behaviour of the individuals kept in aviaries as the general patterns of night activity did not differ in amount or in temporal patterns. These results suggest that the “classical” method of observing migratory activity within cages in captivity is valid and yields similar results to the measurement of “Zugunruhe” in more natural environments. Thus, our study strengthens the conclusions of previous “Zugunruhe” studies that were criticised because they had been obtained under “artificial keeping conditions”, which were suspected to affect migratory behaviour.

We believe that the absence of population differences both in cages and aviaries is a result of environmental effects, as all three populations were housed in the Madrid area, where natural blackcap populations are “migratory”. Future experiments should test populations from different populations under different environmental conditions, e.g. exposing northern migratory populations to conditions characteristic for a sedentary population. This type of experiment, also known as reciprocal transplant experiment, is necessary for testing the “extended threshold model” and identifying the crucial environmental factors. It will be useful for studying real migration movements as it includes the environment as an important factor in the determination of the migratory phenology (Olsson *et al.* 2006, Brodersen *et al.*, 2008, Skov *et al.*, 2010,



Pulido, 2011). This will allow us to make more reliable predictions of evolutionary changes in migration propensity in response to environmental changes.



5.3 Chapter 3

“Are exploration and neophobia associated with migratory behaviour? An experimental study in blackcap (*Sylvia atricapilla*)”

Previous studies had found that resident birds are less neophobic and more exploratory of a novel object within their known environment than migrants (Mettke-Hoffmann and Gwinner, 2004; Mettke-Hoffmann *et al.*, 2005b). Other studies concluded that migrants are fast and superficial explorers with lower spatial neophobia in a novel environment (Mettke-Hoffmann *et al.*, 2009). Fast explorers as well tend to be rigid, less innovative and prone to routine-like behaviour in their response (Verbeek *et al.*, 1994; Verbeek *et al.*, 1996).

Our results are more in line with the latter studies. Birds from the resident population of Tarifa proved to be have shorter latencies in the moderate stress experiment and were the least flexible and the most rigid in adapting feeding behaviour to changes in food location within their cages. An adaptive explanation for these findings could be that residents rely more on their previously learned experiences, as they remain within the same area year-round, in which environmental conditions are relatively constant. In contrast, birds from the Madrid and Cocentaina populations proved to be more flexible in their behaviour, discarding faster the empty, but familiar, food source and directing their attention to the new one. While migrating, foraging on stopover sites is time constricted, thus a more flexible feeding behaviour could be an advantage. The lack of statistically significant differences for variables explaining exploratory/neophobic behaviour does not allow straightforward conclusions. Tarifa birds, however, seemed to have shorter exploring/neophobic latency in the memory test, possibly implying better memory of the food location.

Although we did find some differences in personality traits among populations differing in migratory behaviour, we did not find a correlation between these traits and the amount or timing of migratory activity (“Zugunruhe”) displayed by blackcaps in captivity. Therefore, we cannot conclude that these among-population differences are due to differences in migratory activity. Previous studies obtained contradictory results regarding the correlations between personality traits and migratory activity. This may be attributable to differences in the species and in the experimental design used in the different studies. So it is not clear whether a link between personality traits and migration may generally be expected.



5.4 Chapter 4

“Long-term memory in blackcaps (*Sylvia atricapilla*) differing in migratory behaviour”

The analysis of the data from the six personality experiments, which were conducted in two consecutive seasons, demonstrated long-term memory effects in Iberian blackcaps from the migratory (Madrid) and the partially migratory population (Cocentaina); unfortunately, the sedentary population could not be tested. This effect was found in the latency to feed that significantly decreased from the first experiment in autumn to the last one in spring.

Long term memory effects were first described in parids in a study of black-capped chickadees (*Parus atricapillus*). This species can remember the location of a single food item for at least 6 months, maintaining spatial memories for long time (Roth *et al.*, 2011), ability previously only found in corvids (Balda and Kamil, 1992). Later on, studies have correlated long term memory with migratory propensity, a study on garden warblers (*Sylvia borin*) reported the ability of migratory birds to memorize and remember a particular feeding site for at least a year (Mettke-Hofmann and Gwinner, 2003). A close non-migratory relative, the Sardinian warbler (*Sylvia melanocephala momus*), wasn't able to retain information about certain feeding site for more than two weeks (Mettke-Hofmann and Gwinner, 2003). These findings together make long-term memory an important factor for birds that undergo more than one migration season. It is an important asset in remembering the location of quality stopover sites and migration routes, especially in older birds that use a more complex orientation system based on memory and learning (Mettke-Hofmann and Gwinner, 2003). Surprisingly, this also could hold for the migratory blackcap populations studied here, which compared to the garden warbler, perform more facultative short-distance migratory movements.

The maintenance of latencies from the end of the autumn to the beginning of the spring season indicates that the behaviour learned within the autumn experiments was maintained at least until spring. Hence, we were able to demonstrate the longevity of the response to stress within a period of at least four months.



5.5 Chapter 5

“Dominance in blackcaps (*Sylvia atricapilla*) from three populations differing in migratory behaviour”

We examined possible differences in dominance status between a migratory (Madrid), a partially migratory (Cocentaina) and a sedentary population (Tarifa) of blackcaps. Based on the “dominance hypothesis” (Gautheraux, 1978) we predicted that individuals from sedentary populations would be dominant over migratory individuals. The results of population comparison between Madrid and Tarifa could not confirm this. Contrary to expectations, blackcaps from Madrid tended to initiate interactions (being positively correlated with the weighted dominance) more often than blackcaps from Tarifa. Moreover, the fact that in 11 out of 16 pairwise comparisons, between birds from Madrid and Tarifa, the individuals from Madrid were dominant suggests that in Iberian blackcaps migrants are dominant over residents. This finding may be a consequence of migrants fuelling up for the autumn migratory season (Bairlein, 1985), or higher motivation for early arrival to breeding grounds and territory establishment.

No differences in weighted dominance score or number of initiated or won interactions were found between Cocentaina and Madrid. However, birds from the Cocentaina population tend to be in a subordinate position relative to birds from the Tarifa population. The tendency is supported by the dominance score being negatively correlated with onset of migratory activity (sedentary have later onset, see Chapter 4) and positively with tarsus size. We, therefore, may conclude that in this population comparison, in which the bigger individuals from Tarifa tended to be dominant over birds from Cocentaina, the predictions of body size hypothesis were met (Lindström *et al.* 1990, Dierschke *et al.* 2005).

Individuals with higher dominance score had shorter latencies when approaching the unfamiliar plate (tendency in memory test), implying that dominant individuals tend to be fast explorers (Verbeek 1996?). Moreover, we also found a stronger tendency for keeping old habits (i.e. frequent returning to the usual feeder) in the exploration and memory test, which may suggest that dominant individuals are more rigid and less flexible in adjusting their behaviour to changes in food location, where they apparently rely more on their previous experience (aggressive rodents being more dominant Benus *et al.*, 1991).

Besides exploration, another important factor determining the social hierarchy is the ability of individuals to cope with stress. Fast explorers lost a high dominance status once they found themselves in groups, where they became subordinate to slow exploring individuals (Verbeek, 1999) because slow explorers were able to recover faster from stressful situations (Dingemanse and de Goede, 2004). Our individuals displayed the same change in dominance status from pairs to social groups, where the dominants in pairwise encounters, lost their position on the hierarchy scale (negative linear regression coefficient for the dominant individuals within pairs), caused probably by poor stress coping due to lost interactions (Carere *et al.*, 2001,



2003; Verbeek, 1998; Verbeek et al., 1999). We did not find any correlation between the amount or intensity of migratory activity, as measured in aviaries or cages, and the dominance score.





6 CONCLUSIONS





6.1 Chapter 1

(1) Can we measure differences in the onset and amount of migratory activity in a common-garden experiment in captivity between three populations differing in migratory behaviour in the wild? (Chapter 1)

We were able to measure the two main characteristics of the migratory activity; its onset and amount. However, the displayed results were unexpected as all three populations of blackcaps displayed similar amounts of nocturnal activity during the autumn migration season. However, in contrast to these results, we observed differences in the onset of migratory activity where birds from the migratory Madrid population started autumn migratory activity earlier than birds from the other two populations. We hypothesize that within the Iberian Peninsula among-populations differences in migratory behaviour are primarily due to differences in environmental conditions rather than being genetic. In this region, both resident and migratory populations could find themselves close to the migration threshold, therefore being particularly sensitive to environmental conditions. Housing the birds in a locality with environmental conditions experienced by migratory populations (Madrid) could have shifted the migration threshold, hence, birds from all three populations displayed migratory behaviour.

6.2 Chapter 2

(2) Are there any differences in the migratory activity between two environments; between activity inside cages and aviaries as the more “natural” environment? Is behavioural expression of migratory restlessness different within aviaries compared to cages? (Chapter 2)

Keeping birds in the more “natural” environment, aviaries, had only small effects on the expression of their migratory behaviour and the amount of migratory activity did not generally differ. However, Iberian blackcaps started migratory activity significantly earlier in aviaries when compared to cages. In addition, birds from Tarifa demonstrated higher plasticity by tendency of being more active in aviaries than in cages, possibly due to higher sensitivity to environmental changes, confirming the predictions of the “environmental threshold model”.

Birds’ predominant behaviour inside cages was wing whirring, while flying was displayed in aviaries within all three populations, which represents previously undescribed data. The recorded nocturnal activity in cages was indeed a display of migratory behaviour (i.e. nocturnal flight), not just escape behaviour or a locomotory activity due to possible disturbances. We claim that it corresponds to the flying behaviour of the individuals kept in aviaries as the general patterns of night activity did not differ in amount or in temporal patterns. The difference probably originates from different housing conditions as the caged birds are confined to a much smaller place and unable to display their “natural” flying behaviour during migration. These results suggest that the “classical” method of observing migratory activity within cages in captivity is valid and yields



similar results to the measurement of “Zugunruhe” in more natural environments. Thus, our study strengthens the conclusions of previous “Zugunruhe” studies that were criticised because they had been obtained under “artificial keeping conditions”, which were suspected to affect migratory behaviour.

(3) Do individuals from these populations differ in their nocturnal activity patterns? Is there a measurable difference in night activity patterns between populations? (Chapter 2)

The amount and patterns of night activity differed between the two environments only in the central part of the observed migration season, and only during the first few hours of the night time, when birds in aviaries were more active. However, the general patterns of night activity did not differ in amount or shape of the night activity distribution and there was no population or environment specific pattern of migratory activity. Although the main type of behaviour shown by blackcaps in these two environments differ, the patterns and displayed amounts are similar, and the difference in type is probably only a consequence of different housing conditions.

(4) Is there an influence of environmental variables, specifically temperature and weather conditions, on migratory activity? (Chapter 2)

The migratory activity of individuals kept in aviaries was indeed affected by weather conditions. Meteorological variables, that have been used to characterize “good” or “bad” weather, have been previously identified as one of the most critical proximal cues determining the onset of migratory activity. We did not find any influence on the onset; however, conditions characterised as “bad” weather did cause a general reduction of migratory activity.

6.3 Chapter 3

(5) Is it possible to identify and describe the “personalities” in the blackcap (*Sylvia atricapilla*), from 3 Iberian populations differing in migratory strategy, and determine the behavioural elements which compose them? (Chapter 3)

In this study we found significant population differences in personalities among three populations of Iberian blackcaps. The results suggest that sedentary (Tarifa) population is coping better with moderate stress, possibly an adaptation for staying year-round in an environment with moderate changes. As well, it resulted to be the least flexible population in adapting to changes in food location. An explanation may be within the lack of necessity for innovation in feeding behaviour as they can rely on previous experiences while remaining within the same area all-year round. In contrast, birds from the Madrid and Cocentaina populations proved to be more flexible in their feeding behaviour; while migrating, foraging on stopover sites is time constricted, thus, a more flexible feeding behaviour could be an advantage.



The lack of statistically significant differences for variables explaining exploratory/neophobic behaviour does not allow straightforward conclusions. Tarifa birds, however, seemed to have shorter exploring/neophobic latency in the memory test, possibly implying better memory of the food location.

It is also possible that there are no behavioural differences in exploration to detect within our study species or they are too subtle to be detected. The blackcap is a species in which all three migratory strategies, suggesting rapid evolutionary changes of those strategies in response to the changing environmental conditions. Hence, it is possible that prevailing personality types are the ones more flexible, capable of adjusting to different environmental conditions over relatively short period of time.

(6) Based on the set of novelty experiments, is it possible to explore inter-population differences and see if a generalization of population's personality types could be applied depending on their migratory strategy? (Chapter 3)

Although we did find some differences in personality traits among populations, we did not find a correlation between these traits and the amount or timing of their migratory activity displayed in captivity. Therefore, we cannot conclude that these among-population differences are due to differences in migratory activity. As the previous studies obtained contradictory results regarding the correlations between personality traits and migratory activity, presumably due to differences in the species and in the experimental design used in the different studies, it is not clear whether a link between personality traits and migration may generally be expected.

The lack of a strong correlation between personality types and amount of displayed migratory activity may be due to the artificial experimental environment in which real differences in migratory behaviour may not be expressed, or the housing location of the birds and its environmental conditions, as we assume that the Madrid's migratory latitude could have induced the migratory behaviour within all three populations causing the disappearance of personality types-migratory activity correlation.

6.4 Chapter 4

(7) Is there a long term memory in our subject species? Does the memory of information learned during the autumn migratory season persist until the spring migration period and can this information be used in the corresponding situations? (Chapter 4)

Long-term memory is an important factor for birds that undergo more than one migration season necessary for remembering the location of quality stopover sites and migration routes. The analysis of the data demonstrated long-term memory effects in Iberian blackcaps from the migratory (Madrid) and the partially migratory population (Cocentaina) (the sedentary population could not be tested). This effect was found in the latency to feed that significantly decreased from the first experiment in autumn to the last one in spring. The



maintenance of latencies from the end of the autumn to the beginning of the spring season indicates that the behaviour learned within the autumn experiments was maintained at least until spring.

6.5 Chapter 5

(8) Is it possible to observe the establishment of social hierarchy within pairs of individuals belonging to different populations, based on their pairwise encounters? Is there a correlation between dominance status and migratory activity? Does the position of an individual within a social hierarchy determine whether or not this individual will migrate? (Chapter 5)

We were able to observe the establishment of social hierarchy within paired individuals of different populations. Blackcaps from Madrid tended to initiate interactions (being positively correlated with the weighted dominance) more often than blackcaps from Tarifa. This finding may be a consequence of migrants fuelling up for the autumn migratory season, or higher motivation for early arrival to breeding grounds and territory establishment. No differences in weighted dominance score or number of initiated or won interactions were found between Cocentaina and Madrid. However, birds from the Cocentaina population tend to be in a subordinate position relative to birds from the Tarifa population. We, therefore, may conclude that in these studied Iberian populations, dominance does not play a role in determining their migratory propensity. We did not find any correlation between the amount or intensity of migratory activity, as measured in aviaries or cages, and the dominance score.

(9) Is there a correlation between dominance status and personality type? Are dominant individuals predominantly fast explorers and subordinate individuals slow explorers? (Chapter 5)

Individuals with higher dominance score had shorter latencies when approaching the unfamiliar plate (tendency in memory test), implying that dominant individuals tend to be fast explorers. Moreover, we also found a stronger tendency for keeping old habits (i.e. frequent returning to the usual feeder) in the exploration and memory test, which may suggest that dominant individuals are more rigid and less flexible in adjusting their behaviour to changes in food location, where they apparently rely more on their previous experience.

(10) Does the dominance rank of an individual obtained by interactions with a conspecific change once that individual finds itself in a larger social group? Do dominant individuals retain their superior position or do they become subordinates? (Chapter 5)

Fast explorers lost a high dominance status obtained within housing in pairs once they found themselves in larger social groups, where they became subordinate losing their position on the hierarchy scale, the change probably being caused by poor stress coping due to lost interactions.



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8 Chapter 1

“How the measurement of migratory restlessness under semi-natural conditions can help us gaining a better understanding of the control of bird migration”

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8.1 Introduction

Migration is defined as the regular, endogenously controlled seasonal movement between two distinct areas, one for breeding and the other for non-breeding (Dingle and Drake, 2007; Salewski and Bruderer, 2007) as a response to predictable seasonal changes (Ramenofsky and Wingfield, 2007). In different migratory species it has been demonstrated that migratory birds in captivity show locomotory activity, called migratory restlessness or “Zugunruhe”, that mirrors, to a large extent, the migratory pattern of their conspecifics in wild populations (Berthold, 1973, 1996, 2001; Gwinner and Czelschlik, 1978). When held in captivity, migratory birds display a significant increase in activity around the same time when the free living populations start their seasonal autumn and spring migration (Berthold, 1973, 1990b). The observed migratory restlessness largely corresponds to the migratory behaviour under natural conditions the amount being correlated with the distance the birds travels, duration and directionality of “Zugunruhe” matches the routes taken by birds from the same population in the wild (Gwinner, 1986b; Berthold, 1990a, 1999; but see Helm, 2006). These findings indicate that migratory birds are guided by an innate migration program, which determines the time when to leave the breeding area, with which intensity to migrate, in which direction to go and when to stop migration (Berthold, 1996, 2001). The existence of the endogenous, circannual rhythms has been demonstrated in a number of studies (Berthold and Querner, 1981; Gwinner, 1986b, 1990, 2003). Avian circannual rhythms were first described in migratory willow warblers (*Phylloscopus trochilus*) that were kept under constant photoperiod and temperature for three cycles and still were displaying annual rhythms of moult and migratory activity (Gwinner, 1986a). Most of the attention so far has been focused on studying “Zugunruhe” in migrants, while it was presumed that such phenomenon is not characteristic for residents. Surprisingly, some resident species express intense nocturnal activity during migratory periods (Smith *et al.*, 1969; Chan, 1994; Berthold, 1996; Helm and Gwinner, 2006; reviewed in Helm 2006).

One of the model species for studying migration is the blackcap (*Sylvia atricapilla*) as its populations display large geographic variation in migratory behaviour (Pulido, 2007). The studies by Peter Berthold and his co-workers on hand raised blackcaps kept in Germany from populations ranging from southern Finland (migratory populations) to Canary Islands (sedentary populations) differing in travelled distance, showed genetic differences in migratory restlessness mirroring actual differences in migratory behaviour in the wild (Berthold and Querner, 1981). Hybrids between birds from different populations displayed amounts of “Zugunruhe” which was intermediate between the activities of the parental populations, demonstrating the genetic basis of among-species variation in migratory behaviour (Berthold and Querner, 1981). However, as each of these populations is exposed to different environmental condition (e.g. temperature, day length or food availability) among-population differences in migratory behaviour could also be due to environmental effects. Thus, the migration strategy of each population is a result of a genetic adaptation to the particular conditions



at the areas of reproduction, migration and wintering and the effect of these environmental conditions on the expression of the behaviour (Pulido, 2007).

In order to clarify the mechanism which determine if a bird migrates or is resident, a genetic “threshold model” has been proposed describing inheritance and evolution of migratory behaviour (Pulido *et al.*, 1996). This model is based on an underlying normally distributed continuous variable (e.g. amounts of proteins or hormones involved in migratory activity), called the migration propensity or liability, correlated with migratory activity. The migration threshold converts this continuous variable it into dichotomous variable with two categories (migrant, non-migrant), determining the phenotype of each individual. It is not fixed but is expected to vary individually depending on genetic variation and environment’s influence on that variable. Thus, environments favouring residency will shift the distribution of liability below the threshold and migratory behaviour will not be displayed (Pulido, 2007, 2011). It is observed that populations of long-distance migrants and established populations of residents find themselves further away from the threshold, where environmental effects on the expression of their migratory phenology are minor and do not change migration status (Pulido, 2011).

The aim of this study was to compare migratory activity of populations, which presumably are close to the migration threshold, on a small geographical scale. We, therefore, studied three blackcap populations on the Iberian peninsula, which differ in migratory behaviour in the wild: a migratory population (short distance) from central Spain (Madrid) (Carbonell and Telleria, 1998; de la Hera *et al.*, 2007), a partially migratory population from the central Mediterranean coast (Cocentaina) (Morganti *et al.*, 2015), and a sedentary population from the very south of the Iberian peninsula (Tarifa) (Tellería and Carbonell, 1999; Tellería *et al.*, 2001, Shirihai *et al.*, 2001).

Birds from these populations (phenotypes) would be housed in a “common garden” experimental setup, i.e. under identical controlled environmental conditions which are especially important for the populations around the migration “threshold”, where certain environmental variable could induce or supress the expression of migratory activity (Pulido, 2011), such as the food availability (Terrill, 1987; Gwinner *et al.*, 1988) or nocturnal illumination (Ramenofsky *et al.*, 2008). Hence, with this setup we intended to explore the underlying genetic difference in migratory behaviour (Gwinner, 1969; Berthold, 1990a; Pulido, 2007) of our three test populations based on possible phenotypic differences (van Noordwijk *et al.*, 2006). If we were to find no difference in migratory activity, it would indicate that phenotypic differences do originate from differing environments or different responses to the environment (van Noordwijk *et al.*, 2006), being an additional support for the “threshold model” (Pulido, 2011).



8.2 Materials and methods

The study was done on juvenile blackcaps. The blackcap is a common and widespread warbler, one of the most abundant passerines in the western Palaearctic (Shirihai *et al.*, 2001). This species is a nocturnal migrant with populations expressing all three migratory strategies – long distance migrants, mostly from northern Europe, partial migrants from in southern Europe, and sedentary populations from southernmost Iberia and the Atlantic islands (Berthold, 1996; Shirihai *et al.*, 2001).

Captured juveniles (2-3 months old) belonged to both sexes from 3 Iberian populations: Madrid (Pinilla del Valle 40°55'N, 3°49'W), Cocentaina (Alicante) (38°44'N, 0°26'W) and Tarifa (Los Barrios 36°11'N, 5°36'W). We selected these populations because they allowed us to study differences in migratory strategy on a small geographical scale. Based on observations and ringing recoveries, birds from Madrid population are considered migratory (Carbonell and Tellería, 1998; de la Hera *et al.*, 2007), from Cocentaina partially migratory (Morganti *et al.*, 2015) and from Tarifa sedentary (Tellería and Carbonell, 1999; Shirihai, 2001).

Juveniles were captured in summer of each of 3 consecutive years: 2010 (N=33, mean capture date 25th of June), 2011 (N=33, mean capture date 28th of June) and 2012 (N=22, mean capture date 1st of August). In addition, in 2011, 9 birds from Cocentaina population were hand raised and their migratory activity was analysed. This group of birds allowed us to compare the method we used for determining “Zugunruhe”, i.e. measuring migratory activity in fledglings captured in the wild, with “Zugunruhe” data obtained from hand-raised birds, which is the method most of the studies on nocturnal restlessness have used to minimize the influence of the native environments (Berthold, 1973; Gwinner, 1977; Berthold and Querner, 1981; van Noordwijk *et al.*, 2006). After capture, birds were transported to our study facility in the restricted area within the Madrid's natural park (Casa de Campo), where they were kept in individual cages (45x23x38 cm) with food and water *ad libitum*. Temperatures inside the experimental room followed the natural fluctuation of temperature (minimum temperature of 1.5°C and maximum of 27.9°C for the observation period), but were attenuated by about 4°C (i.e. maximal temperatures were lower and minimal higher than outside). All birds had visual contact with individuals in neighbouring cages, and auditory contact with all individuals. After the experimental period, at the end of May of the year following capture, all individuals were introduced into aviaries to regain flight ability and to feed again on natural food. After removal of colour rings, they were released at the exact sites where they had been captured.

8.2.1 Experimental design

For minimizing environmental variation among individuals, all birds were kept under an identical feeding protocol, light and temperature conditions. This so-called “common garden” approach was applied in order to determine genetic differences in migratory activity among our three populations. Autumn nocturnal activity was recorded from the beginning of September until the end of December and we are considering the



observed increase in nocturnal activity as the display of migratory activity. Each cage was equipped with two movable perches connected to micro-switches. Activity was recorded using a Microscript® recording system (Berthold *et al.*, 1972), where each movement was recorded on a scaled thermic paper roll. Night locomotory activity was measured from 16:00 h to 10:00 h of the following morning, as the total number of perch movements per 30min intervals during the lights out period (Berthold *et al.*, 1972) and calculated as the sums of the 30min intervals with activity (only night activity was analysed). Birds perceived natural daylight through two windows (1x1.5 m). However, as light intensity was low, we gave them additional light (2 compact fluorescent lamps, Megaman WL 130 Compact 2000 HPF, with a power of 30 W, a light intensity of 1620 lumen and a colour temperature of 6500 K=daylight) that followed the natural photoperiodic regime of Madrid. The photoperiod was adjusted weekly around the solstices, and twice a week around equinoxes to mimic natural conditions using the data of sunrise and sunset of the weather station at Barajas, (Madrid, http://www.tutiempo.net/tiempo/Madrid_Barajas/LEMD.htm). An additional night light (2-3 Lux) was mounted centrally on the ceiling of the experimental chamber as migratory activity is not expressed in complete darkness (Helms, 1963). There were short periods of missing data due to the occasional power cuts or malfunction of registration system, these gaps of 1-2 days were either treated as missing data (see below) or replaced by the average value of migratory activity per night from the preceding and following 5 days.

8.2.2 Variables analysis

Variable name	Considered activity	Minimum number of 30min intervals	Period considered
5-day onset of MA	all	≥ 5	at least five consecutive days at the start of the phase with continuous activity
5-day onset of high MA	high	≥ 5	at least five consecutive days at the start of the phase with continuous activity
3 intervals onset of activity	all	≥ 3	first part of the night (22:00-02:00h) at the start of the phase with continuous activity
4 intervals onset of activity	high	≥ 4	first part of the night (22:00-02:00h) at the start of the phase with continuous activity
Continuity independent 3 intervals onset	all	≥ 3	at the start of the phase independently of the continuity of activity

Table 1: all activity; high activity” (i.e. bird constantly active through the entire 30min period)

For the statistical analysis was used IBM’s SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp). PC’s extracted were analysed by a GLIM model stepwise backward analysis. The final model was selected based on the lowest corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC).



We have defined several onset and amount of migratory activity variables in order to avoid any biased results in birds with low or interrupted activity. For that reason, the onset of migratory activity (MA) was defined for each individual as the first Julian date of a period with continuous activity at which the sum of activity per night was equal or higher than five 30 min intervals during at least five consecutive days, (“5-day onset of MA” criterion) (see Pulido *et al.*, 2001a; Pulido and Coppack, 2004). Similar criteria were applied on extraction of the rest of the variables, but including different minimum numbers of activity intervals, all or only “high activity” (i.e. bird constantly active through the entire 30min period) and parts of the dark period (see Table 1 for the summarized description of the variables).

Principal component (PC) analysis for the onset of migratory activity was conducted with all five variables (see Supplementary table 1) and one component was extracted, which explained 73.1% of the variance.

The end of migratory activity was defined as the last day, given in Julian dates, of a continuous 5-day-period with a minimum of five 30min activity intervals. Duration of migratory activity was calculated by subtraction of the onset of MA Julian date from the end of MA Julian date. Variables were described both for the lower and higher activity.

The amount of the migratory activity (MA) was measured as the sum of 30min periods of night activity during the migration season, which was defined as the period between the onset and the end of migratory activity (see above for definition), corrected for the number of days with missing data (MA maximum days of activity). The second variable is a measure of the mean intensity of migratory activity over the complete migration period. It was calculated as the sum of 30min activity periods, excluding days with no activity, divided by the number of nights with activity (MA intensity). Both variables were calculated using migration onset and termination for both all and high activity, and replacing missing data only for the first variable.

Principal component (PC) analysis, using all the variables described above, yielded two components: PC1 (“PC amount”) mainly representing the total amount of activity (72.46% of variance) and PC2 (“PC amount of high activity”) representing the amount of the high activity (20.25%) (loadings in Supplementary table 1).

Only one *Cocentaina* individual did not display continuous migratory restlessness, thus it was excluded from the analyses.



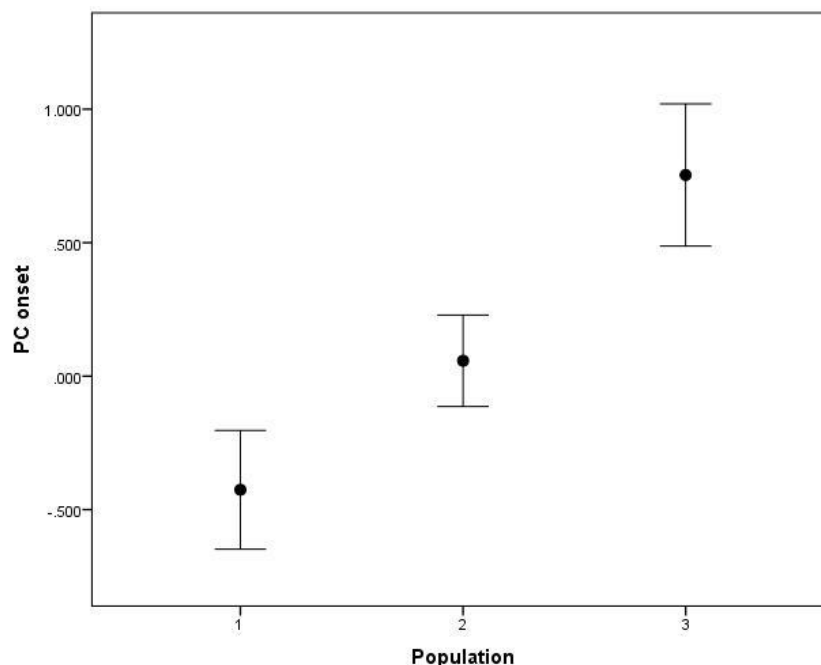
8.3 Results

8.3.1 Comparison of “Zugunruhe” in hand-raised blackcaps and fledglings caught in the wild

No difference was found between the wild-caught juveniles and the hand-raised birds from Cocentaina of the same cohort neither in onset (PC onset $F(1)=0.707$, $p=0.413$), amount (PC amount of activity $F(1)=0.594$, $p=0.452$; PC amount of higher activity $F(1)=3.085$, $p=0.098$) or duration of migratory activity (Duration of activity $F(1)=0.013$, $p=0.910$; Duration of higher activity $F(1)=2.263$, $p=0.152$). The end of the lower activity only displayed a tendency towards difference between the wild-caught juveniles and the hand-raised birds from Cocentaina, while they displayed same mean end of higher activity (End of activity $F(1)=4.377$, $p=0.053$; End of higher activity $F(1)=0.314$, $p=0.582$).

8.3.2 Onset of migratory activity

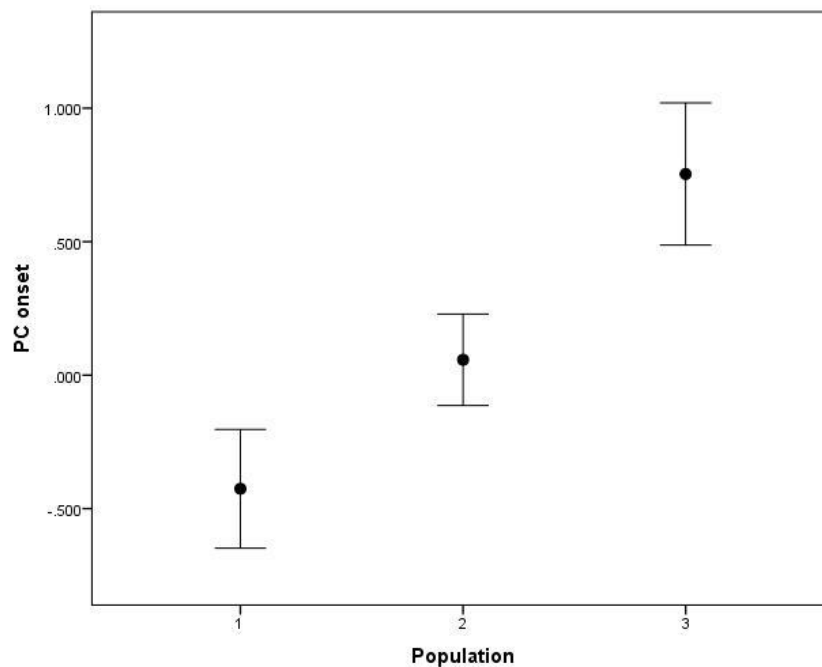
The analysis of the variable “PC onset” showed significant effects of population (Wald $\chi^2=21.906$, $p<0.001$) and cohort (Wald $\chi^2=20.912$, $p<0.001$). In addition, the interaction effects of population-by-sex (Wald $\chi^2=6.138$, $p=0.046$) and cohort-by-sex (Wald $\chi^2=17.896$, $p<0.001$) were significant (Supplementary table 1).



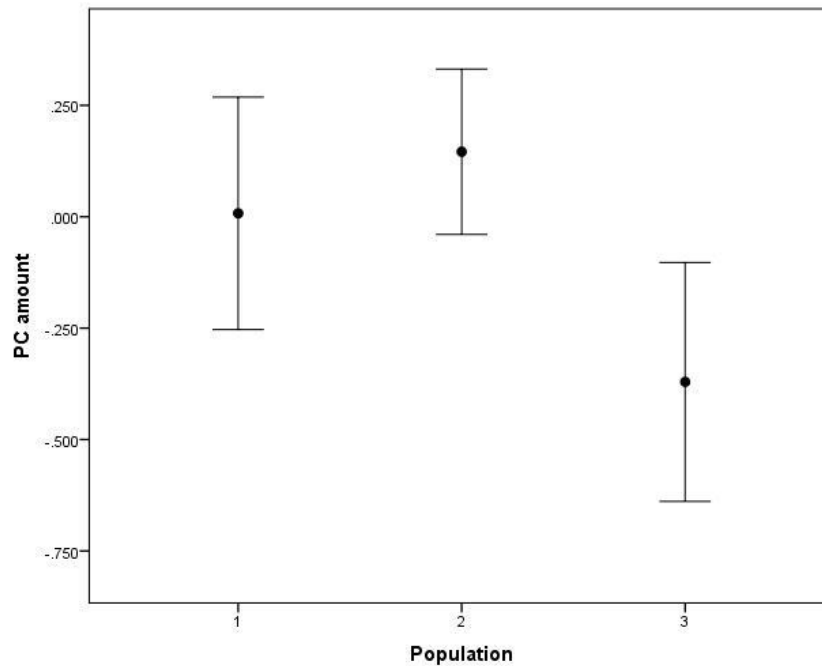
Graph 1: Mean (filled circles) and standard errors of the onset of migratory activity (PC) in three Spanish populations of blackcaps; Population 1-Madrid (N=27); 2-Cocentaina (N=33); 3-Tarifa (N=16).

Analysing the effect of population on the principal component of migration onset showed significant difference between three populations ($F_{(2)}=5.891$, $p=0.005$), the difference in migration onset between Madrid

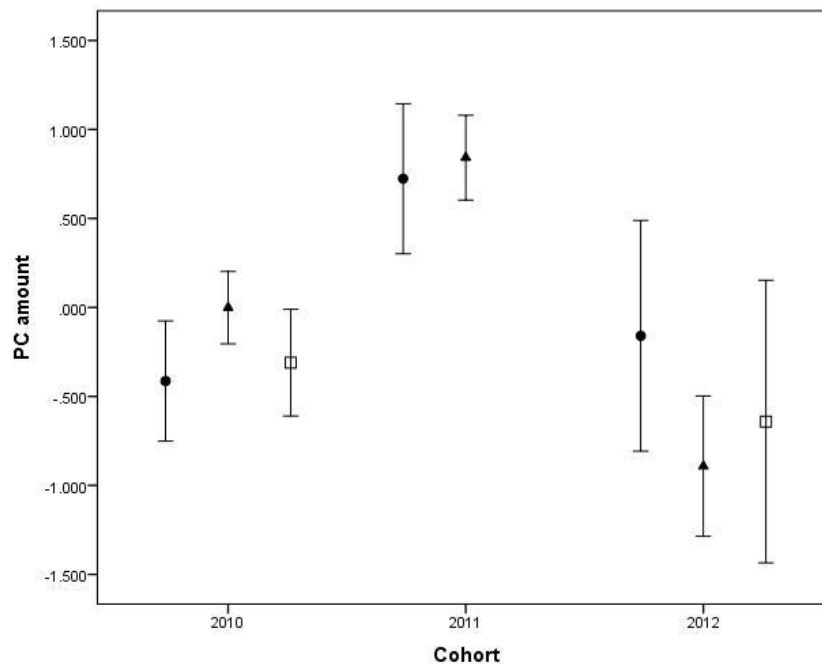
and Tarifa being statistically significant (Tukey HSD $p=0.003$ Madrid with earlier mean onset of -0.43 , Tarifa 0.75). The significant cohort effect ($F_{(2)}=6.326$, $p=0.003$) showed that birds from the 2010 cohort had a significantly later onset compared to birds born in 2011 (Tukey HSD $p=0.004$; mean 2010= 0.42 , 2011= -0.52 , 2012= -0.24). Cohorts 2011 and 2012 were not significantly different (Tukey HSD $p=0.136$). Regarding the population-by-sex interaction effect (described above), males did not differ in mean onset across population ($F_{(2)}=1.345$, $p=0.276$) while females of Tarifa and Madrid are the ones with the latest (mean 1.11) and earliest onset (mean -0.67), respectively ($F_{(2)}=4.725$, $p=0.021$, Tukey HSD $p=0.016$) (Graph 3). In addition, in the significant cohort-by-sex interaction effect, males have similar mean onset across three cohorts ($F_{(2)}=1.383$, $p=0.267$), while females have the latest onset within the autumn of 2010 ($F_{(2)}=8.342$, $p=0.002$, Tukey HSD 2010-2011 $p=0.006$, 2010-2012 $p=0.020$).



Graph 2: Mean (empty symbols) and standard errors of the onset of migratory activity (PC) of males and females from three Spanish populations of blackcaps. Population 1=Madrid (N=27); 2=Cocentaina (N=33); 3=Tarifa (N=16).



Graph 4: Mean (filled squares) and standard errors of the amount of migratory activity (PC) in three Spanish populations of blackcaps; Population 1-Madrid (N=27); 2-Cocentaina (N=33); 3-Tarifa (N=16); displaying no significant difference between populations ($F_{(2,53)}=1.045$, $p=0.359$).



Graph 5: Mean (filled triangles) and standard errors of the amount of migratory activity (PC) in three Spanish populations of blackcaps within the three cohorts; Madrid marked with filled circles (N=27); Cocentaina marked with filled triangles (N=33); Tarifa marked by empty squares (N=16).

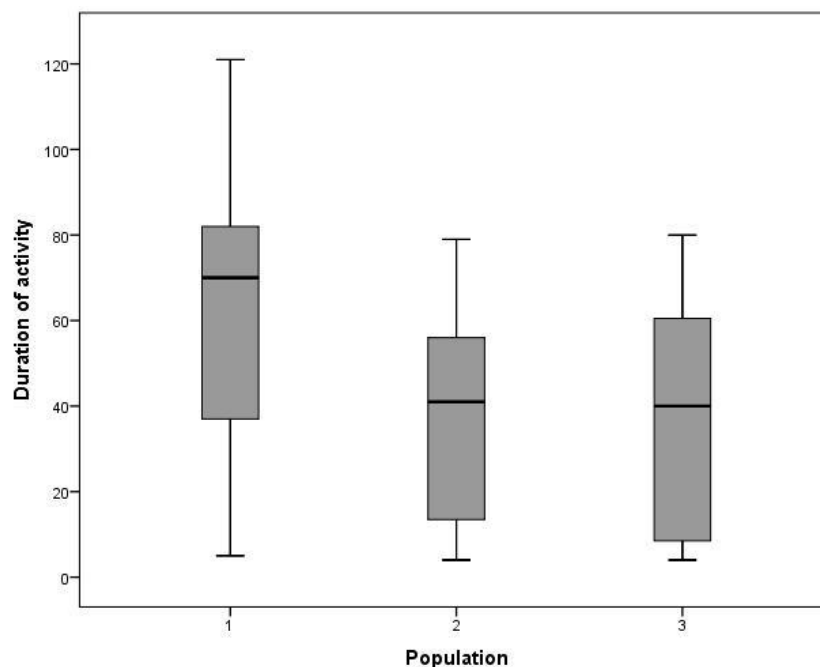
8.3.3 Amount of migratory activity

The model analysis for the variable “PC amount” resulted only in significant cohort effect (Wald $\chi^2=21.907$, $p<0.001$), while “PC amount of high activity” showed none (Supplementary table 1). Significant cohort effect ($F_{(2)}=10.367$, $p<0.001$; Bonferroni 2010-2011 $p=0.001$, 2011-2012 $p<0.001$) is due to the higher activity within 2011 cohort compared to birds from other cohorts (mean 2010=-0.21, 2011=0.79, 2012=-0.62).

In the graph 5 we have separated the “cohort effect” by its associated population compositions in order to examine if the significant difference in cohorts' means is due to the absence of Tarifa population within the second year, but we can see that Tarifa is not causing a decrease in mean value within the other two cohorts containing all three populations (2010 $F_{(2,27)}=0.659$, $p=0.526$; 2012 $F_{(2,7)}=0.511$, $p=0.620$).

8.3.4 Duration of migratory activity

A model analysis of the duration of migratory activity, displayed significant population (Wald $\chi^2=18.106$, $p<0.001$), cohort (Wald $\chi^2=20.535$, $p<0.001$) and population-by-cohort interaction (Wald $\chi^2=13.674$, $p=0.003$) effects. Birds from Madrid had the longest duration of migratory activity (Kruskal-Wallis $\chi^2(2)=6.979$, $p=0.031$, mean rank for Madrid=36.53, mean rank for Cocentaina=24.19, mean rank for Tarifa=24.18). Duration of MA for higher activity displayed no significant factors (Supplementary table 2).

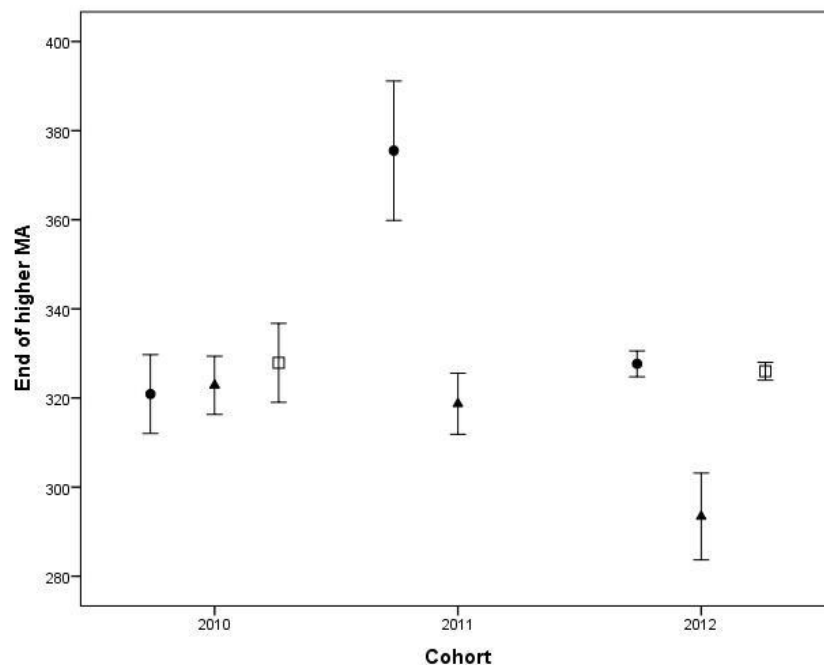


Graph 6: Duration of migratory activity for three Spanish populations of blackcaps expressed in mean Julian date; Population 1-Madrid (N=27); 2-Cocentaina (N=33); 3-Tarifa (N=16).



8.3.5 End of Migratory activity

In general, the analysis of the end of migratory activity yielded no significant effects (Supplementary table 2). However, the end of higher MA showed significant effects of population (Wald $\chi(2)=16.641$, $p<0.001$), cohort (Wald $\chi(2)=15.685$, $p<0.001$) and population-by-cohort interaction effect (Wald $\chi(3)=14.526$, $p=0.002$). Populations differ in their end of MA ($F(2)=3.805$, $p=0.029$) where Madrid ends its MA later compared to Cocentaina (Tukey HSD $p=0.020$). Regarding the found significant population-by-cohort interaction, significant differences can be found within 2011 cohort ($F(2)=14.678$, $p=0.002$), between Madrid and Cocentaina birds. Within the 2012 cohort, only a tendency exists towards difference between Madrid and Cocentaina populations ($F(2)=5.067$, $p=0.044$, Tukey HSD $p=0.060$) (Graph 7).



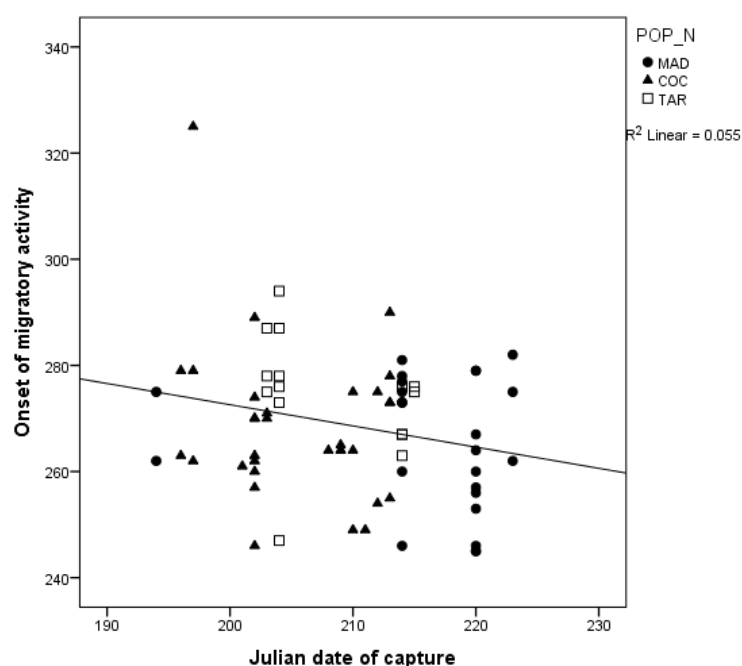
Graph 7: Mean and standard errors of higher migratory activity in three Spanish populations of blackcaps, expressed in mean Julian date; Madrid population marked with filled circles (N=27), Cocentaina population marked with filled triangles (N=33), Tarifa population marked with empty squares (N=16).

8.4 Discussion

A surprising result of this study was that all three populations displayed similar nocturnal activity during autumn migration season. For the sedentary (Tarifa) and the partially migratory population (Cocentaina) this was contrary to the behaviour of blackcaps from these populations in the wild. While we did not find among-population differences in the proportion of migrants or the amount of migratory activity displayed, we found differences in the onset of migratory activity. This difference could be reflecting



migratory phenology of the free living populations, as the more northern migrants should leave earlier for their autumn migration to cover the longer distance to the wintering grounds. On the other hand, difference in the onset of migratory activity might have been due to among-population differences in hatching date of our experimental birds. Generally, there is a strong positive correlation between hatching date and onset of autumn migratory activity. In the blackcap, for every two days a chick hatches later it starts its migratory activity one day later (Pulido *et al.*, 2001a; Pulido *et al.*, 2001b; Coppack *et al.*, 2001). As our birds were caught as juveniles, we do not know their exact hatching date. Thus, a potential difference in mean hatching of birds collected in different populations may have caused the observed difference in onset of autumn migration. However, Madrid population also demonstrated a significantly longer duration and later end of migratory activity, compared to the other two populations. As both earlier onset and longer duration are characteristics of more migratory bird populations it is unlikely that observed differences are only due to the possible among-population variation in hatching date.



Graph 8: Correlation between date of capture and onset of migratory activity for all three cohorts pooled (“5-day onset of MA”), Spearman’s $r = -0.116$, $p = 0.336$. Assuming that we caught all juveniles at the similar age, the absence of significant correlation gives us an indication of the onset effect found due to genetic differences rather than differences in hatching date.

The fact that all three populations displayed similar amounts of migratory activity is novel and requires another explanation. We have to mention that the observed discrepancy in our results with the literature cannot be attributed to the difference in the method using juveniles instead of the hand-raised birds (i.e. the usual protocol), as there were no differences in the migratory behaviour between the two groups of the same cohort. Expression of migratory activity within resident population is not unexpected, as some resident species express intense nocturnal activity during migratory periods (Smith *et al.*, 1969; Chan, 1994; Berthold, 1996; reviewed in Helm and Gwinner, 2006). However, nocturnal migratory restlessness found in resident white-



crowned sparrows (*Zonotrichia leucophrys*) (Mewaldt *et al.*, 1968), blackcaps (*Sylvia atricapilla*) (Berthold, 1996), or resident stonechats (*Saxicola torquata*) (Helm and Gwinner, 2006) was expressed in significantly lower levels than in migratory species or populations, which is in discrepancy with findings of our study. Variation in the expression of migratory activity has a strong genetic component (Berthold, 1996; Pulido and Berthold, 2003; Pulido, 2007), however, the environment plays an important role in its inhibition or release (van Noordwijk *et al.*, 2006; Helm, 2006; Pulido, 2011). According to the environmental threshold model of migration, “migration genes” are present in all individuals, however, environmental variables might change the expression of migratory activity for the individuals close to the migration threshold, determining whether an individual is a migrant or a resident (Adriaensen *et al.*, 1990; Berthold, 1984; Lundberg, 1988; Pulido, 2011). Until recently, it was not considered that migratory activity in captivity could be influenced by environmental factors but the photoperiod (Berthold, 1979, 1988, 1996; Gwinner, 1986b). Factors that usually affect migratory propensity in the wild, such as food availability, competition, temperature, latitude and weather conditions or density (Boyle, 2011; Newton, 2008) were considered negligible and not taken into account (but see Merkel, 1938; Ketterson and Nolan, 1987; Terrill, 1987; Fusani *et al.*, 2011). Only recently it has been accepted that those factors may modify migratory behaviour, at least of some captive species, with their intensity varying along migratory season, between or within populations (see revision in Pulido, 2011).

Hence, housing of all three populations in one locality (Madrid), with environmental conditions experienced by migratory populations (Tellería *et al.*, 2001) may have been the main cause for migratory activity in captive birds not reflecting migratory behaviour in the wild. Small differences in latitude (which entails differences in magnetic field), photoperiod, humidity and temperature, compared to Tarifa and Cointin, might be sufficient for changing the expression of migratory activity of the partially migratory population inducing migratory activity in birds that are resident in their populations of origin. Having in mind the species’ geographic variation in migratory behaviour and the possibility of its rapid adjustments to climate changes (establishment of a new wintering area, Berthold and Terrill, 1988), we might consider observed migratory activity as a flexible response of the partially migratory and resident populations to poor wintering conditions at the Madrid location.

How is this flexibility enabled? As proposed by the environmental threshold model of migration, individuals with extreme values of the continuous variable, such as long-distance migrants or resident populations, will be further away from the threshold and largely insensitive of the influence of the environmental variation on their migratory phenotype (Pulido, 2011); being “environmentally canalized” (Pulido and Widmer, 2005). If environmental conditions change, resident populations may adapt because they preserve genetic variation in migratory traits (cryptic genetic variation). It is presumed that resident populations closer to the threshold may have particularly large cryptic variation, enabling rapid adaptation to conditions in novel environments by expression of the migratory phenotype (Schlichting, 2008; McGuigan and Sgrò, 2009).



Our results are at odds with Peter Berthold's studies on blackcap populations ranging from migratory populations in southern Finland to residents from Canary Islands, that showed distinct migratory restlessness for each population related to their natural travelled distances, with hybrids displaying intermediate values, demonstrating the genetic basis of migratory behaviour within this species (Berthold and Querner, 1981). Similar results were obtained in experiment on stonechats from Kenya, Siberia and central Europe, which were kept under synchronising natural day length in a laboratory in Germany. These birds showed increasing differences in amount of migratory activity between populations ranging from residents to long distance migrants (Helm, 2006). The reason for the discrepancy between these results and the results from our study on Iberian blackcaps could be due to their variation at a much smaller geographical scale, the Iberian Peninsula, including short distance migrants, partial migrants and residents. We hypothesize that due to their geographical origin these populations do not represent clearly the differentiated migratory strategies, and could find themselves closer to the migration threshold, and therefore be particularly sensitive to environmental conditions. Transfer to and maintenance of these blackcaps under "Madrid" conditions could have shifted the threshold to the left, causing expression of the migratory activity in all three populations. For confirming this crucial environmental influence on the observed result, it would be interesting to house birds of these three populations in Tarifa, under the environmental conditions that do not induce migratory activity in the wild. Under these conditions we would expect Tarifa birds not to display "Zugunruhe" and birds from Cointine and Madrid to repress their migratory activity.



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8.6 Supplementary material

	Onset PC	Amount PC 1 PC 2	
% of variance	73.10	72.46	20.25
Eigenvalue	3.657	4.348	1.215
Factor loadings			
5-day onset of MA	0.780		
5-day onset of high MA	0.875		
3 intervals onset of activity	0.948		
4 intervals onset of activity	0.838		
Continuity independent 3 intervals onset	0.826		
MA maximum days of activity		0.901	-0.410
MA intensity		0.839	-0.445
MA maximum days of higher activity		0.877	0.442
MA intensity of higher activity		0.706	0.574
MA maximum days of activity (filled gaps)		0.894	-0.426
MA maximum days of higher activity (filled gaps)		0.874	0.378

Table 1: Factor loadings and % of explained variance for the principal component factor analysis of the onset of migratory activity and amount of the migratory activity; PC 1 all activity extracted variables, PC 2 “high activity” extracted variables; for the autumn seasons only of the 2010-2013 period

	Starting model	Final model (p<0.05)						
	AICC, BIC	AICC, BIC	Population	Cohort	Sex	Population*sex	Population*Cohort	Cohort*Sex
PC onset	147.8; 165.4	139.7; 155.7	<0.001	<0.001	ns	0.046	ns	<0.001
PC amount of activity	165.3; 183.4	148.2; 155.5	ns	<0.001	ns	ns	ns	ns
PC amount of higher activity	188.5; 206.6	164.8; 172.1	ns	ns	ns	ns	ns	ns
Duration of activity	535.4; 553.1	521.6; 535.6	<0.001	<0.001	ns	ns	0.003	ns
Duration higher amount of activity	728.8; 752.7	714.8; 729.0	ns	ns	ns	ns	ns	ns
End of activity	689.7; 713.9	669.8; 678.3	ns	ns	ns	ns	ns	ns
End of higher activity	533.5; 547.5	605.9; 623.3	<0.001	<0.001	ns	ns	0.002	ns

Table 2: Summary of the model selection explaining variation in migratory activity. Corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC) were used to find the final GLIM model, p values for each of the factors included in the final model. Significant effects are stated by its p value, ns stands for a non-significant effect.





9 Chapter 2

“Migratory behaviour in Iberian Blackcaps (*Sylvia atricapilla*) supports the environmental threshold model of migration”

Bulaic M, van Heusden J, Morganti M, Ramírez Á, Aguirre JI, Pulido F (unpubl. manuscript)





9.1 Introduction

It has been demonstrated for a number of species that migratory birds in captivity show a significant increase in activity around the same time when the free living populations initiate their seasonal migration (Berthold, 1973, 1996). This activity, called migratory restlessness or “Zugunruhe”, largely reflects the migratory pattern of the wild populations (Berthold, 1973; Gwinner and Czelschlik, 1978; Berthold 2001), and is particularly correlated with the timing, distance and direction of migration of birds under natural conditions (Gwinner, 1986; Berthold, 1990; 1999; Eikenaar *et al.*, 2014).

In obligate migrants, different components of migration, including timing, distance and direction are primarily controlled by a genetic program resulting in relatively constant departure and return to the breeding areas (Berthold 1996, Newton, 2012). A more environment-dependent type of migration is facultative (partial) migration. Birds with this type of migration will overwinter in the same areas they reproduce if environmental conditions are favourable, but will migrate if conditions are unfavourable (Terrill and Able, 1988; Berthold 2001; Newton, 2012). Rather than completely different categories of migration behaviour, we can consider obligate and facultative migration as different states of the same continuous variable (Newton, 2012), where genetic predisposition for migration exists in both categories, but with different levels of environmental canalization (Pulido and Widmer, 2005; Pulido, 2011). In order to clarify the mechanisms which determine whether a bird migrates or not, the threshold model of migration has been proposed, describing inheritance and evolution of the incidence and amount of migratory behaviour (Pulido *et al.*, 1996). This model assumes that there is an underlying normally distributed continuous variable which is correlated with migratory activity and which could be controlling, for example, the levels of proteins or hormones involved in migratory activity. The model further assumes that there is a threshold which transforms this continuous variable into a dichotomous phenotype. Individuals with no migratory activity are residents, with its variable value below the threshold, and migratory if the variable is above the threshold. Laboratory experiments in blackcaps proved that this model accurately describes the incidence of migration and its evolution (Pulido *et al.*, 1996; Pulido and Berthold, 2010)

As all experiments testing this model in birds have been conducted in the laboratory, under artificial conditions, the question has been arisen if the model is generally valid, and particularly whether it is applicable in natural bird populations. In response to these questions, and considering observations of the incidence of migratory behaviour in the wild, an extension of the threshold model was proposed, where environmental factors were considered. This environmental threshold model assumes that, in addition to a genetic predisposition for expressing migratory behaviour, environmental variability determines whether an individual develops migratory activity or not (Berthold, 1984; Adriaensen *et al.*, 1990; Pulido, 2011). This model predicts that individuals at both extremes of the distribution (i.e. with no or high activity) are not sensitive to environmental factors, while those close to the threshold can easily be tipped over to one side or the other, depending on the conditions. Hence, changes in migratory activity depend not only on the



genetically determined liability and the position of the threshold but also on environmental variables. Several environmental factors modifying the propensity to migrate or to remain sedentary have been proposed, such as food availability, temperature and dominance (reviewed by Pulido, 2011). The effect of food availability on migration probability was reviewed for some fish species (Chapman *et al.*, 2012; Dodson *et al.*, 2013), e.g. a roach study where fish migration depended on their somatic state (starvation) and migratory phenotype (Brodersen *et al.*, 2014). However, studies on birds are rare and the contribution of environmental factors in modifying the migration threshold has not been properly assessed yet. Moreover, the importance of other extrinsic (e.g. magnetic field) and intrinsic (e.g. animal personalities) factors is unknown.

Until now, most of the attention was focused on studying “Zugunruhe” in migratory species or populations, while it was presumed that such phenomenon is not found in residents. However, a number of studies suggest that migratory restlessness is not unique to migrants. Some resident species display intense nocturnal activity (Smith *et al.*, 1969; Chan, 1994; Berthold, 1996; Helm and Gwinner, 2006;) during the migratory periods. However, the nocturnal migratory restlessness studied on sedentary populations was expressed in lower levels than in migratory species (Mewaldt *et al.*, 1968; Berthold, 1996; Agatsuma and Ramenofsky, 2006; Coverdill *et al.*, 2011). The behavioural characteristics of the observed nocturnal restlessness were described within some species; in blackcaps it is defined as displays of intense seasonal nocturnal activity within captivity, consisting of hopping, climbing, flying and wing whirring while not leaving the perch or side of the cage, described as “flying with the brakes on” (Gwinner and Czelschlik, 1978; Berthold and Querner, 1988; Berthold *et al.*, 2000). The typical beak-up and beak-up flight behaviour and quiescent phase has been described for resident and migratory white crowned sparrows in captivity (Agatsuma and Ramenofsky, 2006; Coverdill *et al.*, 2011, respectively) This behaviour is believed to represent the gathering of information, looking at the night sky, and taking off for migratory flight.

As all previous studies on “Zugunruhe” have been done measuring migratory activity inside individual cages, we wanted to investigate migratory behaviour under more natural conditions (aviaries), allowing birds to fly and exposing birds to variation in environmental factors with the aim of describing the characteristics of the migratory restlessness within these semi-natural conditions. In addition, we wanted to test predictions of the “extended” threshold model (Pulido, 2011). This model stresses the importance of the environmental conditions each individual experiences for the display of migratory activity, resulting in its expression (migration) or inhibition (residency).

Hence, we tried to integrate environment factors into the experimental study of migratory behaviour by exposing birds inside aviaries to conditions similar to those they would experience within their natural population. Potential differences in migratory activity between a migratory, a partially migratory and a sedentary population would reflect the environmental influence on their migration phenology, and would allow us to draw inference on among-population differences in reaction norms. We presume that migratory populations should show smaller behavioural differences in response to the environmental conditions than the



resident and partially-migratory populations. According to the threshold model, their behaviour in cages, in aviaries and in the wild should be similar, as by the threshold model, they find themselves away from the threshold, and, therefore, should be less sensitive to environmental factors. On the other hand, we expect that birds from sedentary populations, and particularly, partial migrants should show more variation in the expression of behaviour among environments, i.e. have more plasticity, and should show a stronger response to specific temperature and weather conditions within them.

9.2 Materials and methods

9.2.1 Capture and handling

We have captured juvenile blackcaps (*Sylvia atricapilla*) at an age of 2-3 months in three Iberian populations. The population selection was based on different migratory strategy: Madrid (Pinilla del Valle 40°55'N, 3°49'W) representing a migratory population, Cocentaina (38°44'N, 0°26'W) a partially migratory and Tarifa (Los Barrios 36°11'N, 5°36'W) a sedentary population (Tellería *et al.*, 2001). Juveniles were captured in summer of 3 consecutive years (2010, 2011 and 2012), however, only individuals from 2012 (N=36) were used in this study. All birds were trapped between the end of June and mid-July. After capture, birds were transported to our study facility in a restricted area within the Madrid's natural park (Casa de Campo) (40°25'N, 3°45'W), where they were either kept in individual cages (45x23x38cm) with two movable perches, feeder and two drinkers, or aviaries (3x2x2.3m), equipped with 6 perches in three corners, two feeders, two water plates, a natural undergrowth and overhanging holm oaks (*Quercus ilex*), with food and water *ad libitum* daily replenished (fresh fruit of the season, mealworms, industrial food for insectivorous birds "Raff" with addition of vitamins). 16 birds (Madrid N=5, Cocentaina N=4, Tarifa N=7) were kept in outdoor aviaries where they were exposed to the natural photoperiod and temperature fluctuations (range: 1°C-40°C in the sun) and which allowed them to see part of the sky, facilitating the development of the star compass and the use of sunset information (Wiltschko and Wiltschko, 1988a; Spina *et al.*, 1995). The other 20 individuals were housed in indoor cages (Madrid N=5, Cocentaina N=8, Tarifa N=7), under temperature with $\pm 4^\circ\text{C}$ difference to the outdoor temperature conditions. Birds indoors received natural daylight entering through two windows. To reach outdoor light intensity, the room was illuminated with two additional lights (2 compact fluorescent lamps, Megaman WL 130 Compact 2000 HPF, with a power of 30 W, a light intensity of 1620 lumen and a colour temperature of 6500 K=daylight), which were adjusted to the natural photoperiod weekly, mimicking natural conditions (initial 13L:11D; final 10L:14D; weather station at Barajas, Madrid). As migratory activity is not expressed in complete darkness (Helms, 1963) a night light (2-3 Lux) was mounted centrally in the experimental chamber, which was switched on throughout the experiment.

Upon capture, each bird was banded with a colour ring that had a unique code, which allowed us to easily identify it without capture. Individuals inside cages had visual contact with nearby individuals and auditory contact with the rest, while inside aviaries only auditory contact was possible.



9.2.2 Migratory activity

To determine the migratory activity of individuals, nocturnal activity was recorded from the 12th of September until the 14th of November of 2012, which represents the main autumn migration period. Each cage was equipped with micro switches under two movable perches that are connected to the Microscript® recording system (Berthold *et al.*, 1972). Night locomotory activity was recorded from 16:00 until 10:00h of the following morning, as the total number of perch contacts per half-hour intervals during the lights out period (Berthold *et al.*, 1972) and calculated as the sums of the half-hour intervals with activity (only night activity was analysed). Due to the occasional power cuts or malfunction of micro-switches there were short periods of missing data, which were filled with activity means of the five days before and after the day or period without data. All birds were exposed to the natural photoperiodic regime of Madrid. Night locomotory activity for the birds inside aviaries was recorded for the same period, by filming activity with vigilance cameras in infrared mode installed within each aviary from 18:00 until 09:00h of the following morning. One bird (*Cocentaina*) was excluded from the analyses of the onset and amount of migratory activity due to too low activity. Duration of the autumn migratory season was not analysed because the migratory season was not completed (observation ended in mid-November). As migratory patterns differ along the migratory season and it has been known that the peak hour of nocturnal activity can change, we divided our 63 days of observation period to three periods of similar length; the beginning (1st period; the 12th of September until the 1st of October), the middle (2nd period; the 3rd of October until the 21st of October) and the end of the observed migratory period (3rd period; the 23rd of October until the 14th of November). Videos of the night activity in aviaries were analysed by the same observer analysing videos of every other night of the recorded period, where the first half of the each night hour was analysed, by watching 2min for every beginning of the hour and the following 10min periods (4 in total within the half hour periods), assigning scores of 0 for no activity, 1 for activity observed in at least two 10min periods, and 2 for constant activity within the observed period.

9.2.3 Behaviour

To determine the behaviours displayed by captive blackcaps, individuals housed in aviaries (16 individuals) and cages (9 individuals only as the rest were used for dominance experiments) were videotaped throughout the study. Aviary activity was recorded from the 12th of September to 14th of November of 2012, while behaviour inside cages was analysed for activity in spring of 2013 (15th of February to 5th of March of 2013) due to the data availability. Migratory activity behaviour within aviaries was filmed in order to describe and quantify migratory behaviour in captivity, while migratory behaviour in cages was filmed to show that it was a true display of migratory restlessness, not just a night activity due to noise of the migratory neighbours or other factors that could cause night activity. Observed night behaviour was assigned by its frequency to one out of three categories: 1-hopping on the perch; 2-wing whirring; 3-flying. Perch hopping was considered as migratory behaviour, as usually it was displayed shortly before taking off and flying. The analysis of behaviours inside cages was done for a shorter period of time because they were released into aviaries before



ending migratory activity. In cages too, three categories of behaviour were distinguished: 1-hopping on the perch; 2-wing whirring; 3-jumping around the cage while wing whirring.

9.2.4 Variable extraction

9.2.4.1 Onset of migratory activity

The onset of migratory activity (MA) was defined for each individual as the Julian date at which the sum of the night activity was equal or higher than five 30min intervals during at least five consecutive days, at the start of the phase with continuous activity (5-day onset of MA criterion) (see Pulido and Coppack, 2004). Four more variables were extracted for the onset of migratory activity applying different criteria (Table 1), and for more details see Chapter 1.

Principal component analysis for the onset of migratory activity included all the four created variables (PC onset, 76.2% of variance), where the second principal component was extracted from two onset variables of the lower activity (PC onset act1 75.5%), as the data on higher activity were scarce (Madrid N=5, Cocentaina N=5, Tarifa N=5).

Variable name	Considered activity	Minimum number of 30 min intervals	Considered period
5-day onset of MA criterion	all	≥ 5	at least five consecutive days at the start of the phase with continuous activity
5-day onset of high MA criterion	high	≥ 5	at least five consecutive days at the start of the phase with continuous activity
3 intervals onset of activity	all	≥ 3	first part of the night (22:00-02:00h) at the start of the phase with continuous activity
4 intervals onset of activity	high	≥ 4	first part of the night (22:00-02:00h) at the start of the phase with continuous activity

Table1: The four extracted variables for the onset of migratory activity and their criteria described.

9.2.4.2 Amount of migratory activity

Amount of the migratory activity (MA) was measured as the sum of 30 min periods of night activity during the migration season which was defined as the period between the onset and the end of migratory activity corrected for the amount of days with recorded activity in order to account for missing data (MA maximum days of activity). The second variable describing mean MA intensity was calculated as the sum of 30 min activity periods excluding days with no activity (MA intensity). Third variable described the total sum of half-hour activity periods for the entire observational activity period (Total sum of 30 min activity), and the



fourth one was defined as the total sum of half-hour activity periods but excluding the “noise”-corrected for days with only one or two activity periods (Corrected sum of 30 min activity).

PC analysis for the amount of migratory activity was done with all four created variables for lower level of activity (no sufficient data for the higher amount of activity) with one component extracted; PC amount of activity (96.4% of variance) (loadings in Supplementary table 1) (Madrid N=5, Cocentaina N=7, Tarifa N=7).

9.2.4.3 Type of behaviour

The variable used in the analysis of the behaviours within aviaries and cages was obtained by the type of displayed activity per night (1, 2 or 3) weighted by its total number of half-hour intervals when it was displayed as the dominant (most frequent) behaviour.

9.2.4.4 Temperature and weather effects

Temperatures were measured both inside the experimental room with cages, and outside at the aviaries. Minimum outer temperature was used for the analysis, divided into two categories; 1-everything below mean minimum temperature of 8°C for the observation period, 2-all of the above 8°C. Weather was observed as well during the 3 months period, where the night conditions were assigned to two categories: 1-clear skies, 2-everything else e.g. strong wind, clouds, rain.

9.2.5 Statistical analyses

All statistical analyses were carried out using IBM's SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp) and the significance level of $p < 0.05$ was used in interpretation of all obtained results. PC's extracted were analysed by a GLIM model stepwise backward analysis, the final model being selected based on the lowest corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC).



9.3 Results

9.3.1 Onset of migratory activity in autumn migratory season

The four extracted onset describing variables showed no difference in onset between individuals housed in cages or aviaries, except the “4 intervals onset of activity” showing earlier onset within aviaries ($U=8.000$, $p=0.011$). Thus, the general result was that individuals from different populations did not differ in their onset of migratory activity, irrespective of whether they were kept in cages or aviaries. Furthermore, we did not find any effect of housing conditions on the onset of migratory activity for any of the population. These results were generally confirmed by the analyses of principal components extracted for all four onset variables, which indicated no significant population or environment effects. However, the analysis of “PC onset act1” revealed a significant treatment effect on migration timing (ANOVA $F_{(1,29)}=6.159$, $p=0.019$), where birds inside aviaries started migratory activity earlier than birds kept in cages (mean onset aviaries -0.11 ; mean onset cages 0.47). This effect was also significant in the final GLIM model using “PC onset act1” (Wald $\chi_{(1)}=6.584$, $p=0.010$; Table 1), i.e. the model with the lowest BIC.

	Initial model	Final model (Omnibus test $p<0.05$)	Population	Environment	Population*Environment
	AICC, BIC	AICC, BIC			
PC onset	60.4; 57.0	53.5; 53.5 (0.081)	0.053	ns	ns
PC onset act1	76.8; 81.9	66.6; 70.0	ns	0.010	ns
5-day onset of MA criterion	258.9; 265.9	255.6; 261.3 (0.068)	0.058	0.067	ns
5-day onset of high MA criterion	199.3; 203.5	191.1; 194.9 (0.460)	ns	ns	ns
3 intervals onset of activity	274.2; 280.2	266.9; 271.5 (0.155)	ns	ns	ns
4 intervals onset of activity	137.9; 134.5	133.1; 131.8	0.036	0.041	ns

Table 2: Table of AICC (corrected Akaike information criterion) and BIC (Bayesian information criterion) values as analysis selection criteria for the final GLIM model and p values (0.05) for the migratory activity onset variables, with stated factors included in the models. Significant effects are stated by its p value, ns stands for a non-significant effect with p values higher than 0.1.

9.3.2 Amount of migratory activity during the autumn season

None of the four variables extracted reflecting the amount of migratory activity showed significant environment effects (MA maximum days of activity $F_{(1,33)}=2.360$, $p=0.134$; MA intensity $F_{(1,33)}=0.461$, $p=0.502$, Total sum of 30 min activity $F_{(1,33)}=2.020$, $p=0.165$; Corrected sum of 30 min activity $F_{(1,33)}=1.739$, $p=0.196$). Moreover, we found no within-environment differences between the populations studied. In a separate within-population comparison between two environments, only Tarifa individuals showed tendencies of being more active inside aviaries than cages (MA maximum days of activity $F_{(1,12)}=4.525$, $p=0.055$; Total sum of 30 min activity $F_{(1,12)}=4.185$, $p=0.063$; Corrected sum of 30 min activity $F_{(1,12)}=4.637$, $p=0.052$).



The principal component, which integrates variation in all four variables for the amount of activity, yielded the same results: no overall environment or population effects on PC1, and, for birds from Tarifa a tendency for higher activity within aviaries compared to individuals inside cages ($F_{(1,12)}=4.231$, $p=0.062$).

	Initial model AICC, BIC	Final model (Omnibus test $p<0.05$) AICC, BIC	Population	Environment	Population*Environment
PC amount of activity	112.4; 199.1	102.7; 105.4*	ns	ns	ns
MA maximum days of activity	384.5; 391.2	375.2; 377.9*	ns	ns	ns
Corrected sum of 30min activity	385.2; 391.9	376.1; 378.9*	ns	ns	ns
Total sum of 30min activity	383.5; 390.2	373.9; 376.6*	ns	ns	ns

Table 3: Table of AICC (corrected Akaike information criterion) and BIC (Bayesian information criterion) values as analysis selection criteria for the final GLIM model and p values (0.05) for the amount of migratory activity variables, with stated factors included in the models. Significant effects are stated by its p value, * stands for intercept only models, ns stands for a non-significant effect with p values higher than 0.2.

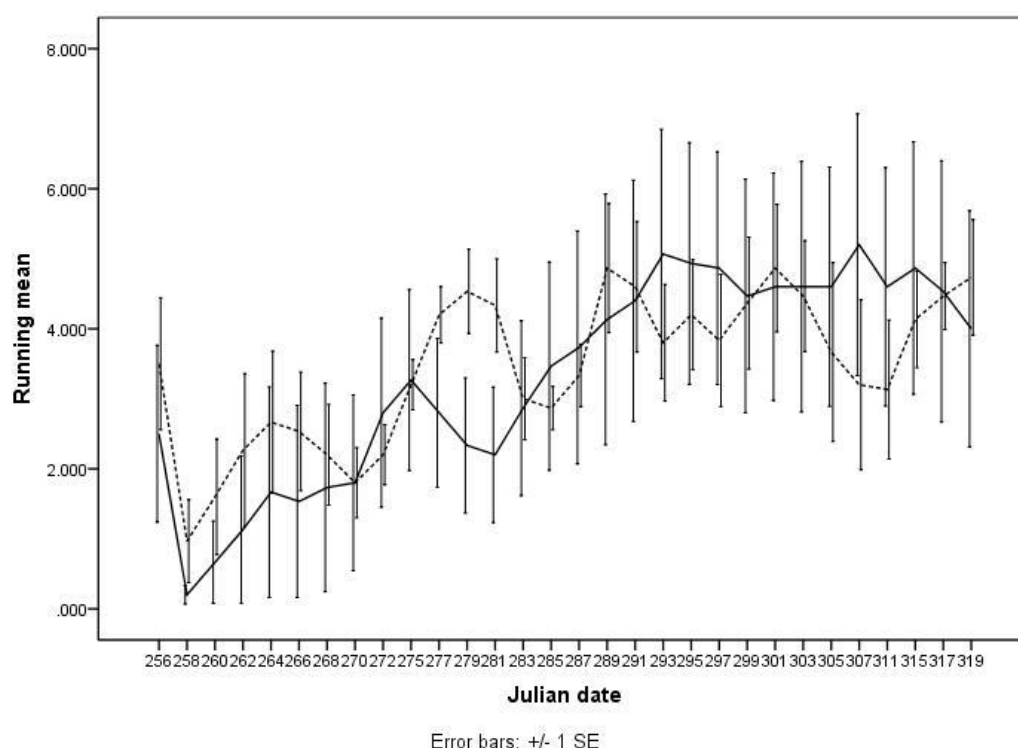


Figure 1: 3-day average running mean of activity for Madrid individuals inside aviaries in broken line and cages in straight line. MA over the observation period does not statistically differ for Madrid individuals inside cages, while inside aviaries temporal variation in activity is statistically significant ($F_{(29,121)}=1.734$, $p=0.021$).

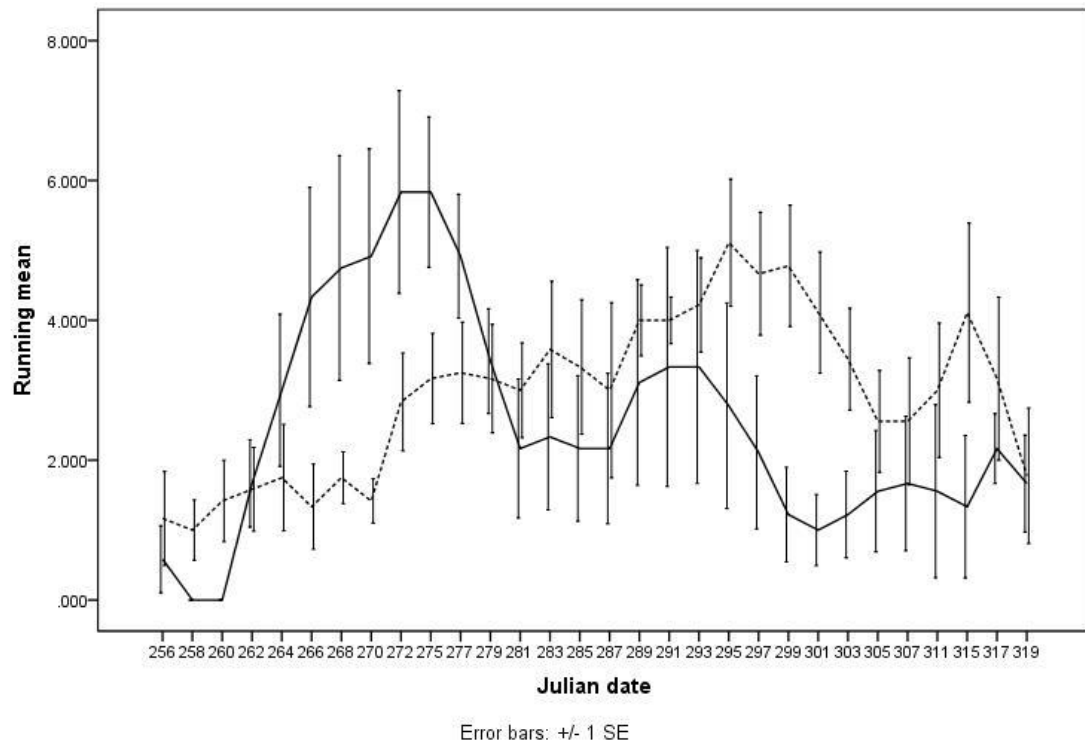


Figure 2: 3-day average running mean of activity for Cocentaina individuals inside aviaries in broken line and cages in straight line.

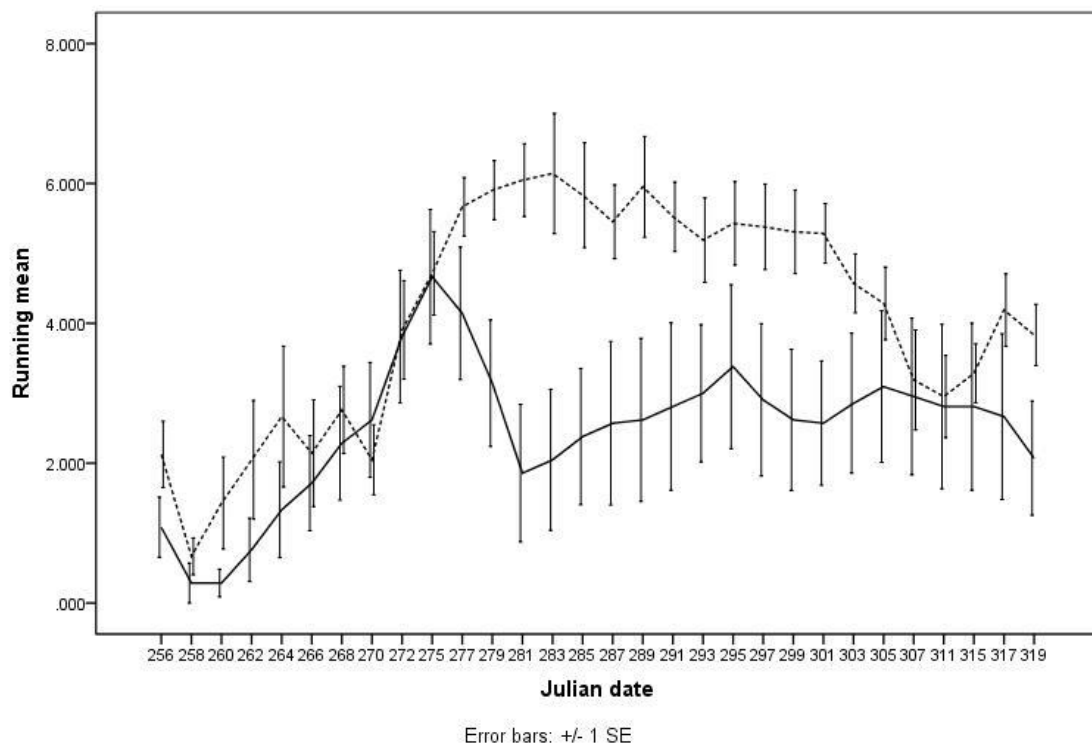


Figure 3: 3-day average of activity for Tarifa individuals inside aviaries in broken line and cages in straight line. Significant difference in MA over time was only observed inside aviaries ($F_{(29,181)}=6.933$, $p<0.001$).



9.3.3 Relation between migration onset and migratory activity

Individual migration onset (PC onset) plotted against the amount of migratory activity (PC amount) gave us a negative correlation between the onset and the amount of displayed migratory activity inside aviaries (linear regression coefficient $B=-0.322$, $p=0.043$), while it was not significant for the individuals inside cages ($B=-0.235$, $p=0.390$) (Figure 4). A full model analysis including the onset of MA as a covariate, including the effects of environment and population and their interactions (Table 4), revealed a significant effect of migration onset on the amount of MA (Wald $\chi_{(1)}=12.719$, $p<0.001$). Moreover, this analysis confirmed the previously described population-by-environment interaction effect, which is due to the higher activity of birds from Tarifa inside aviaries. In none of the populations, we found a significant correlation between onset and amount of activity for individuals kept inside cages (Figure 5). In birds studied in cages, neither pooling the data of all cohorts ($r=-0.082$, $B=-0.082$, $p=0.554$, $N=55$), nor analysing them separately (2010 $r=0.086$, $B=0.089$, $p=0.652$, $N=30$; 2011 $r=0.082$, $B=0.065$, $p=0.762$, $N=16$; 2012 $r=0.243$, $B=0.276$, $p=0.528$, $N=9$) provided evidence that there was a correlation between these two variables (Figure 5).

	Initial model AICC, BIC	Final model AICC, BIC	Population	Environment	Onset(PC)	Pop*Onset	Pop*Envir	Envir*Onset	Pop*Onset*Envir
Migratory activity (PC)	95.72; 89.23	72.67; 76.41	0.036	ns	<0.001	ns	0.007	ns	ns

Table 4: Model selection using corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC) for explaining variation in migratory activity (PC). P values for each of the factors included in the model (Pop=population, Envir=environment) are given for the final model. P values are given for significant effects; ns stands for a non-significant effect. Extracted principal components (PC) were used as variables for the onset and amount of migratory activity.

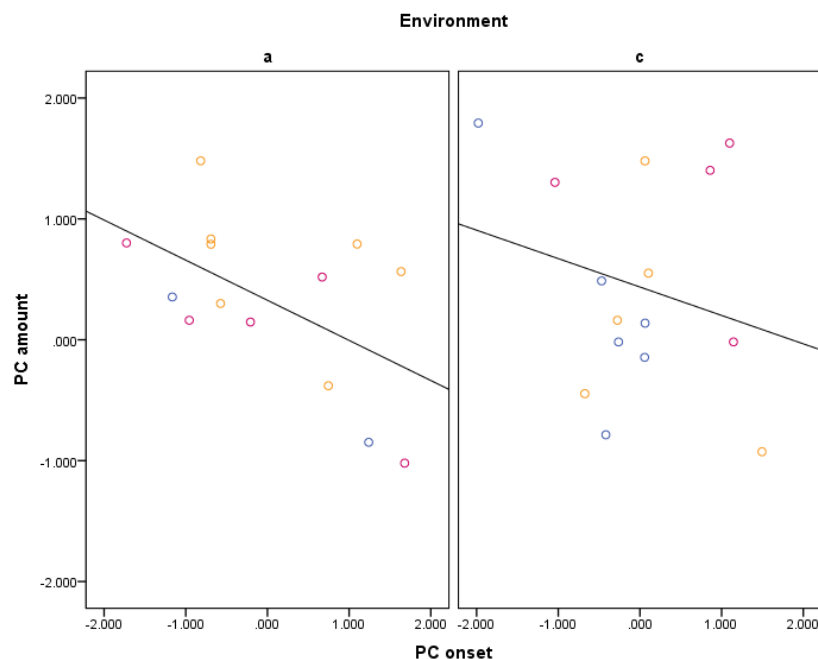


Figure 4: Onset of migratory activity (PC) plotted against the amount of migratory activity (PC) in two environments: (a) individuals housed inside aviaries ($R^2=0.298$, $p=0.043$), (c) individuals kept in cages ($R^2=0.057$, $p=0.390$). Madrid population-blue circles, Cocentaina-purple circles, Tarifa-orange circles.

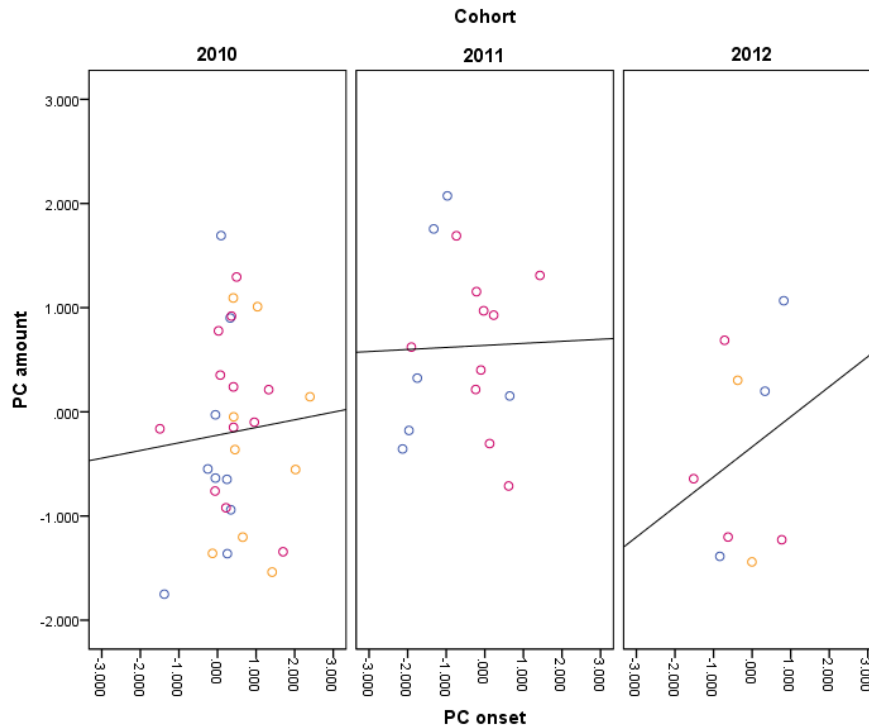


Figure 5: Onset of migratory activity within cages (PC onset) plotted against the amount of migratory activity (PC amount) separately for the three studied cohorts: 2010 ($R^2=0.007$, $p=0.652$), 2011 ($R^2=0.007$, $p=0.762$), 2012 ($R^2=0.059$, $p=0.528$); Madrid population=blue circles, Cocentaina=purple circles, Tarifa=orange

9.3.4 Seasonal peak activity

Mean Julian dates of the peak activity did not differ between the two environments ($F_{(1,34)}=0.998$, $p=0.325$). No differences were found when comparing three populations within each of the environments (aviaries $F_{(2,13)}=2.477$, $p=0.123$; cages $F_{(2,17)}=1.095$, $p=0.357$) or the populations between two environments (Madrid $F_{(1,8)}=0.025$, $p=0.879$; Cocentaina $F_{(1,10)}=0.007$, $p=0.935$; Tarifa $F_{(1,12)}=2.089$, $p=0.174$).

9.3.5 Nocturnal activity and within season pattern

Amounts of activity during night period change over the light off hours, both within cages (Madrid $F_{(11,316)}=1.816$, $p=0.050$; Cocentaina $F_{(11,316)}=4.656$, $p<0.001$; Tarifa $F_{(11,316)}=10.620$, $p<0.001$)(Figure 6) and within aviaries (Madrid $F_{(11,326)}=1.035$, $p=0.415$; Cocentaina $F_{(11,319)}=5.334$, $p<0.001$; Tarifa $F_{(11,326)}=2.070$, $p=0.022$)(Figure 7), with night activity for most individuals peaking around midnight (11 birds). In other individuals (16 birds) maximum activity was displayed towards the end of the night phase. The rest of individuals could not be placed into any of these two categories. In the pooled data for both environments, we find that most of the Cocentaina individuals are more active towards dawn (Pearson's $\chi^2_{(6)}=33.965$, $p=0.001$). The ample sizes are too low to look for environment effects on the peak activity.

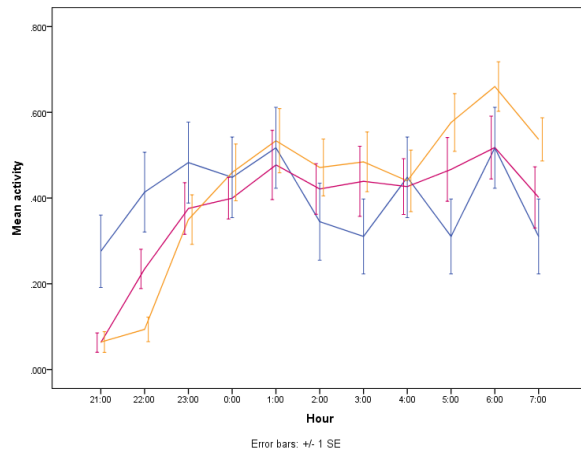


Figure 6: Mean plus standard error activity for the observed period over the light off hours for individuals kept in cages, (September-November; initial 11L:13D, final 10L:14D). Madrid blue line, Cocentaina purple line, Tarifa orange line.

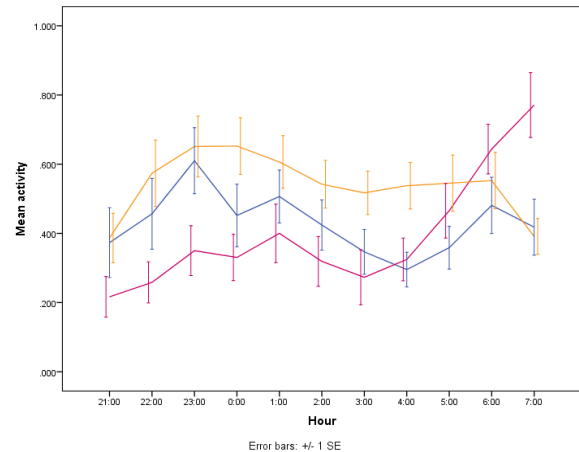


Figure 7: Mean and standard error of locomotory activity during the night hours for individuals kept in aviaries, (September-November; initial 11L:13D, final 10L:14D), Madrid blue line, Cocentaina purple line, Tarifa orange line.

As changes in the nocturnal pattern along the migratory season are likely, we looked into each population's nocturnal activity patterns during three phases: the beginning, middle and the end of the observed period. We found that populations differ in the amount of the activity displayed over the night hours at the beginning (1st period) and at the end (3rd period) of the observed migratory season (1st period: $H_{(2)}=18.787$, $p<0.001$, mean rank Mad=129.6, Coc=177.8, Tar=139.5; 3rd period: $H_{(2)}=77.419$, $p<0.001$, mean rank Mad=226.1, Coc=113.4, Tar=197.3).

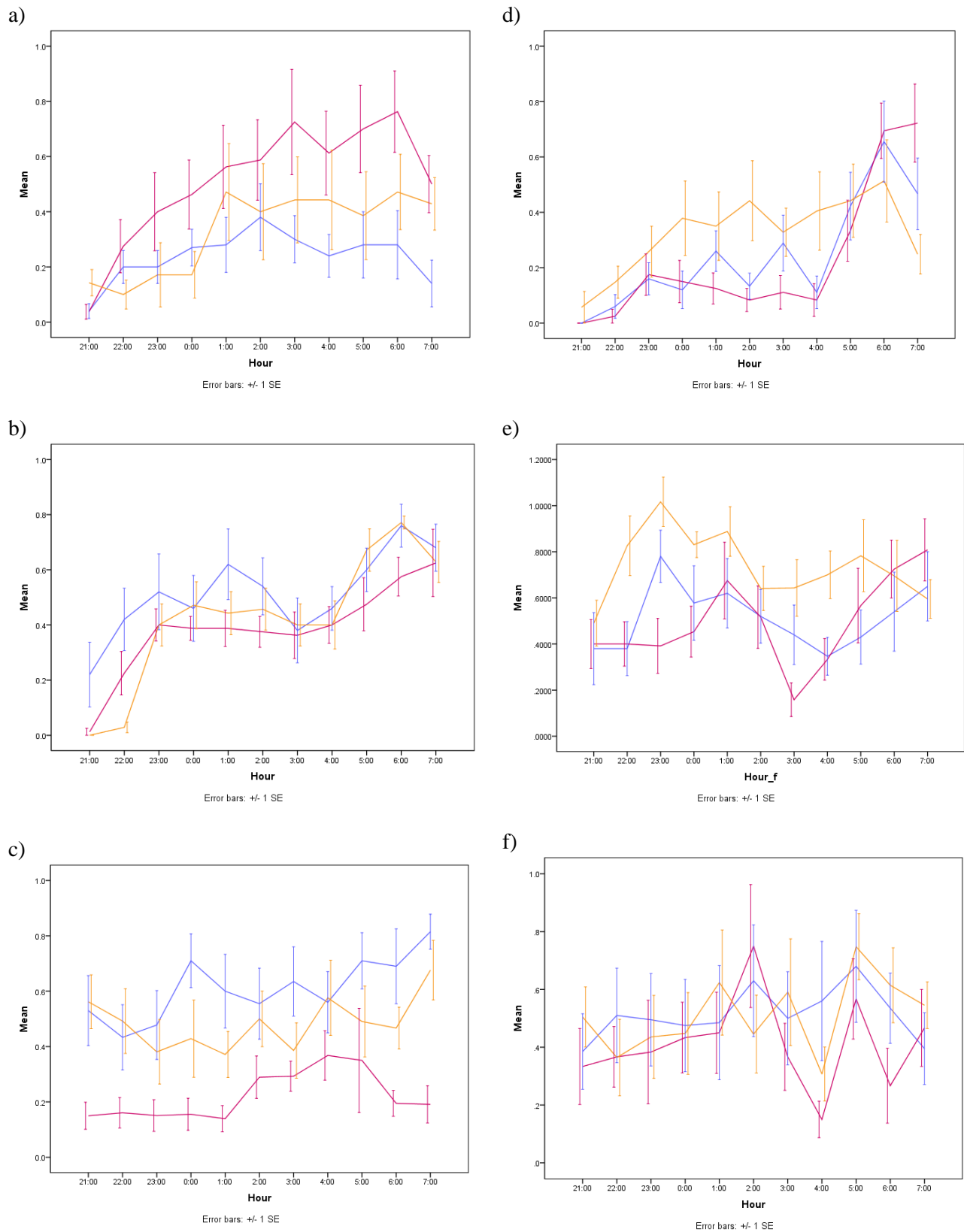


Figure 8: Average activity per hour (sum of 30 min activity per dark phase; 21:00-7:00) for the observed migratory season period for cages; a) period 1, b) period 2, c) period 3. Activity per hour for aviaries; d) period 1, e) period 2 and f) period 3; Madrid dotted line, Cocentaina straight line, Tarifa broken line



For birds kept in aviaries, we found a statistical significant difference in in the amount of nocturnal activity for the 2nd period (Kruskal-Wallis H test, $\chi^2_{(2)}=25.859$, $p<0.001$, mean rank Mad=148.2, Coc=144.1, Tar=202.4) and a tendency for among population differences for the first and third period (1st period: Kruskal-Wallis H test, $\chi^2_{(2)}=5.856$, $p=0.054$, mean rank Mad=159.7, Coc=149.0, Tar=177.9; 3rd period: Kruskal-Wallis H test, $\chi^2_{(2)}=5.519$, $p=0.063$, mean rank Mad=183.2, Coc=159.9, Tar=189.3)(Figure 8).

Within each of the three periods, a comparison of mean nocturnal activity was made between two environmental conditions. The difference proved to be statistically significant only within the 2nd period-the middle of the observed migratory season where individuals inside aviaries were more active than the one in cages, observed during the first half of the dark phase (Figure 9) (1st period Mann Whitney U=45047.0, $p=0.151$; 2nd period U=47248.0, $p=0.004$, 3rd period U=52757.0, $p=0.621$).

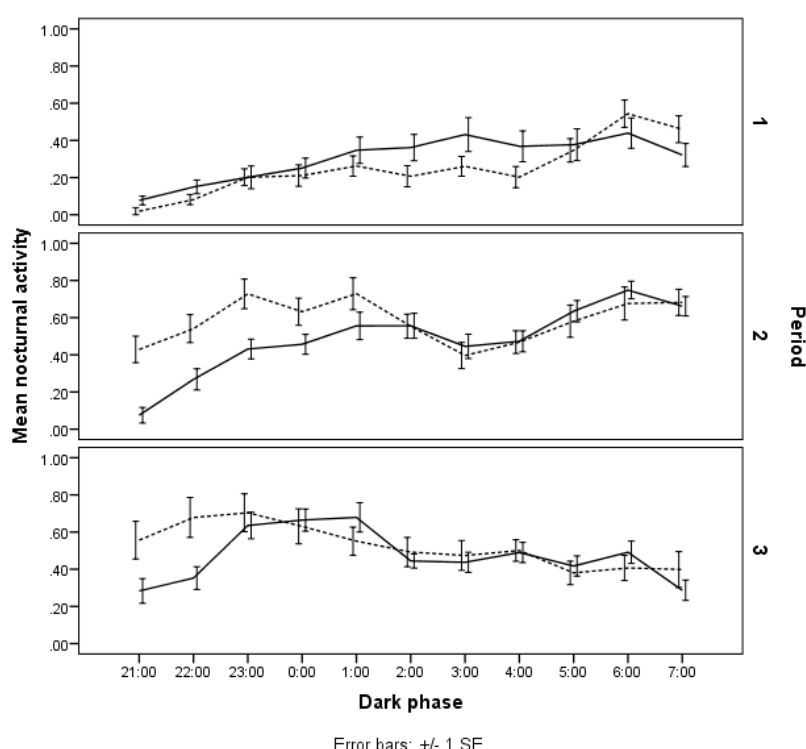


Figure 9: Mean and standard error of locomotory activity per hour during the dark phase (sum of 30min activity per hours of dark phase per date; 21:00-7:00) for birds kept in cages (orange line) and aviaries (violet line) within periods 1, 2 and 3

When analysing population differences between environments in the three periods (Figure 10), only Cocentaina displayed difference in means of nocturnal activity between cages and aviaries within the first part of the migratory activity observation period (Mann Whitney U=3471.5, $p<0.001$). In the second part Tarifa displayed higher activity within aviaries (Mann Whitney U=3710.0, $p<0.001$). Towards the end of migratory season, both Madrid and Cocentaina demonstrated difference in mean activity, Madrid being more active inside cages (Mann Whitney U=5018.0, $p=0.036$) and Cocentaina inside aviaries (Mann Whitney U=4777.5, $p=0.008$).

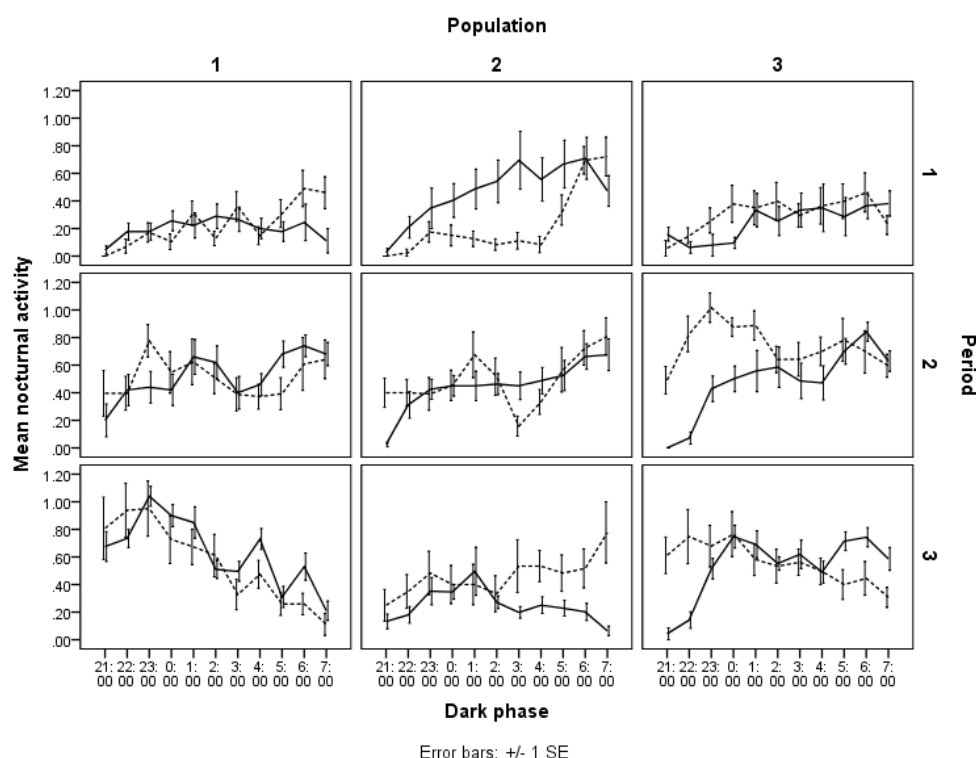


Figure 10: Mean plus standard error of average activity per hour during the dark phase (sum of 30 min activity per hours of dark phase per date; 21:00-7:00) for birds kept in cages (orange line) and aviaries (violet line) within periods 1, 2 and 3, demonstrated separately per populations; Madrid=1, Cocentaina=2, Tarifa=3

9.3.6 Individual activity patterns

Individual activity profiles of night activity revealed high variability among individuals of the same population within both environments. Most birds would start nocturnal activity immediately at the beginning of the dark phase, but some birds would not. An increase in activity would usually happen between 22:00 and 23:00h. Peak activity differed depending on the activity pattern of the individual. For example, in the case of the “bell like” curve the activity pattern reaches its peak around 0:00-1:00h and slowly decreases towards dawn. Another pattern could be described as the “concave” curve with another increase of activity towards dawn. In the third one, activity gradually increased towards the end of the night period. The fourth pattern is similar to the “bell like” curve, but with a sudden increase in activity towards dawn.

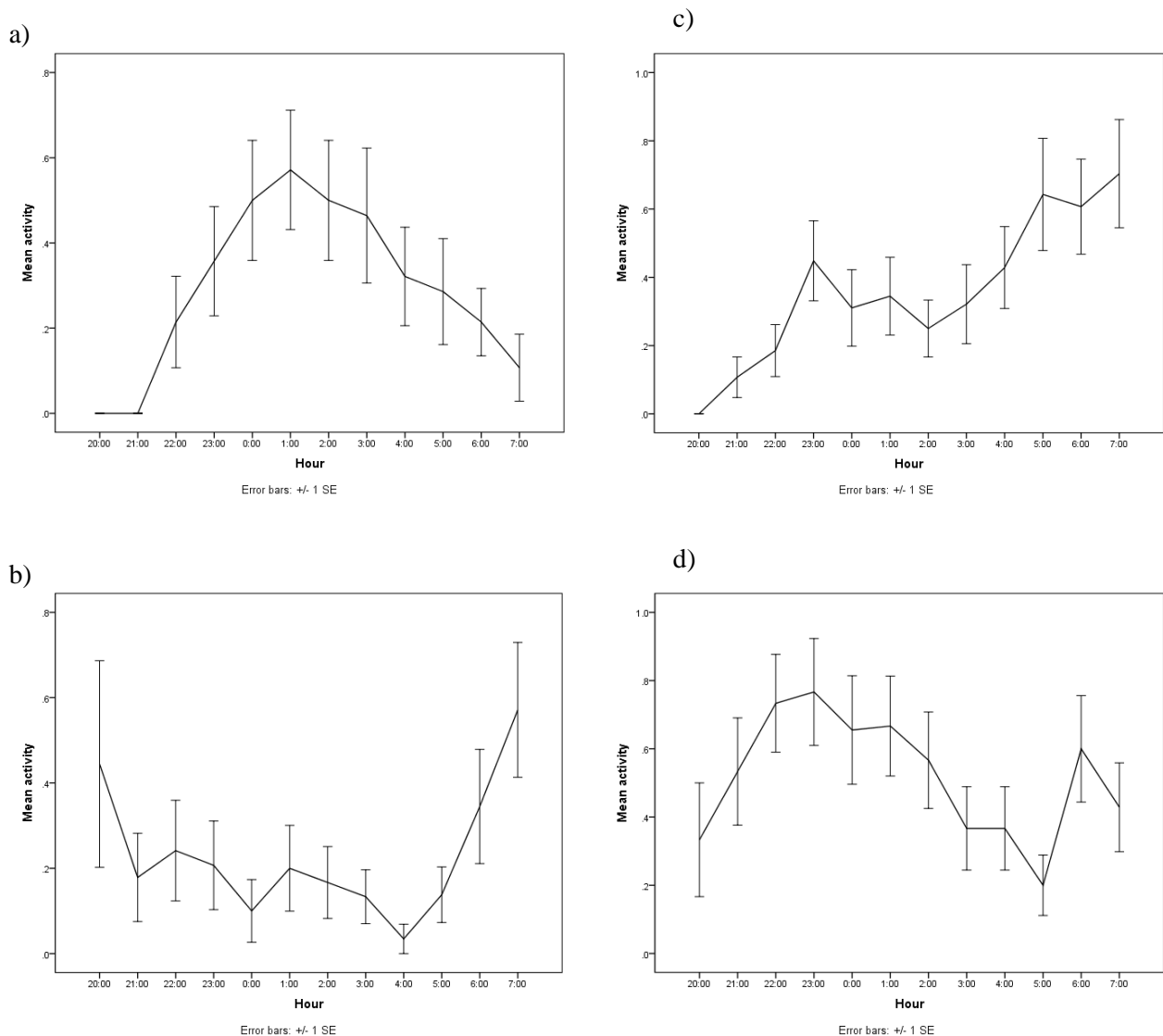


Figure 11: Different categories of individual night activity patterns; a) “bell like” curve reaches its peak around 0:00-1:00h and slowly decreases towards dawn; b) “concave” curve with increase of activity once more towards dawn; c) activity gradually increases towards the end of the night period; d) similar to the “bell like” curve, but with a sudden increase in activity towards dawn.

Chi square test for the type of pattern by population ($\chi_{(6)}=5.952$, $p=0.429$) in general or within each environment (aviaries $\chi_{(6)}=5.608$, $p=0.468$; cages $\chi_{(6)}=6.240$, $p=0.397$) was not significant. In addition, independently of populations, one or the other type of patterns doesn’t prevail in neither of the two environments ($\chi_{(3)}=0.531$, $p=0.912$). It can be seen from the graphs (Supplementary Figure 1) that within each population-environment combination different patterns are present, with ones being more or less frequent, however, there is no population or environment specific pattern.

9.3.7 Type of behaviour inside cages

Analysis showed difference in the percentages of the type of displayed behaviour ($\chi^2_{(2)}=60.272$, $p<0.001$). However, it is due to the fact that none of the nine individuals was displaying hopping (1) as a predominant behaviour, while two individuals displayed wing whirring only (2), one jumping around the cage while wing whirring (3), and the rest was exhibiting combination of the latter two. Although the analysed activity for cages was recorded in spring and sample size is low due to availability, we still find the typical migratory restlessness behaviour within caged birds. Type of activity displayed, independently of population, was positively correlated with the amount of activity; birds with more active periods per night were more likely to display wing whirring while hopping (Kendall's tau b $r=334$, $p<0.001$, $N=82$).

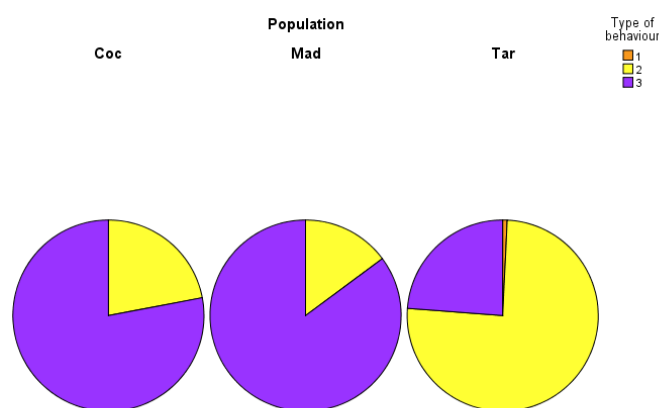


Figure 12: Sums of intervals of each type of activity per night for the 1 month observation period of the activity inside cages, 1- hopping along perch, 2- wing whirring, 3- jumping around the cage while wing whirring; Coc-Cocentaina, Mad-Madrid, Tar-Tarifa.

9.3.8 Type of behaviour inside aviaries

Most of the birds were highly active, except one individual not included in the analysis of the onset and amount of migratory activity because of low activity, but was displaying hopping and flying behaviour. The majority of birds were expressing the same type of behaviour throughout the night and season, while only few of them would interchange from one type to another, like displacements and flying or hopping and flying. Out of sixteen individuals, thirteen (81%) were displaying only flying as the dominant behaviour, while the rest would interchange through flying, wing whirring or hopping during the studied period. All three types of behaviour differed in the amount displayed between populations (total sum of the displayed behaviour per night). Thus, flying behaviour ($\chi^2_{(2)}=17.838$, $p<0.001$) was the most frequent type within Madrid (mean sum of intervals per night of this type of activity for the observing period 238.3) and Tarifa (237.6), while least present within Cocentaina (175.8). The amount of wing whirring behaviour was as well different between populations ($\chi^2_{(2)}=8.250$, $p=0.016$), with the highest mean rank within Tarifa (230.7), then Cocentaina (227.2)



and the lowest within Madrid (213.5). Hopping along the perch was mostly displayed just before taking off and displayed the most within Cocentaina population (261.9), following Tarifa (233.1) and Madrid (188.1) ($\chi^2_{(2)}=36.253$, $p<0.001$). Overall, flying was the behaviour that birds from all three populations displayed most frequently during their nocturnal restlessness in aviaries ($\chi^2_{(2)}=552.548$, $p<0.001$, mean sum of intervals per night of this type of activity for the observing period for hopping 575.69, wing whirring 472.46 and flying 946.46). In addition, the type of activity was positively correlated with the amount of activity; birds with higher sum of active periods per night were predominantly displaying flying behaviour, marked with the higher score (Kendall's tau b $r=0.324$, $p<0.001$).

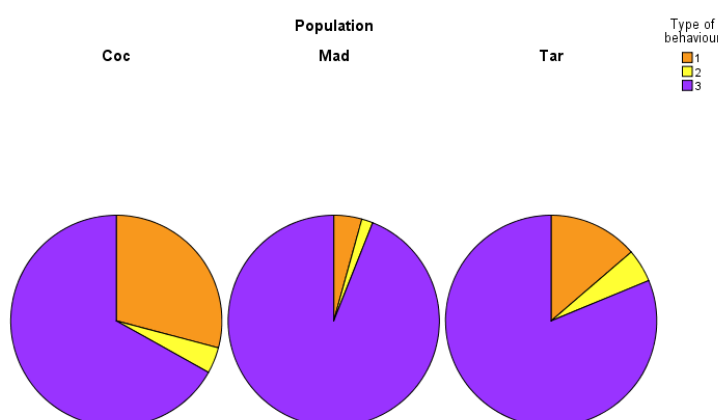


Figure 13: Sums of intervals of each type of activity per night for the 3 month observation period of the activity inside aviaries, 1- hopping along perch, 2- wing whirring, 3- flying; Coc-Cocentaina, Mad-Madrid, Tar-Tarifa

9.3.9 Effect of temperature and weather conditions on the migratory activity

Temperature and weather effects were studied only in individuals kept in aviaries (considered activity after the onset within each individual) as only these birds were exposed to the external weather conditions. The activity variable had no normal distribution, thus the Poisson distribution was the best fitting one. Initial model had included population as factor and minimum temperature conditions (2 categories; 1-everything below mean minimum temperature of 8°C for the observation period, 2- values above 8°C); main effects of all variables and population-by-weather interaction. In the final model (Table 5) temperature had no influence on the amount of activity (Wald $\chi^2_{(1)}=1.827$, $p=0.176$). A Mann Whitney tests showed no difference as well in the amount of activity under different temperature conditions ($U=18649.0$, $p=0.257$).

In the weather effect analysis, the initial model included population and weather conditions as fixed factors (2 categories; 1-clear skies, 2-everything else e.g. strong wind, clouds, rain), main effects of all variables and population-by-weather interaction. In the final model (Table 5) weather proved to have an influence on the amount of activity (Wald $\chi^2_{(1)}=24.630$, $p<0.001$). A Mann Whitney tests showed as well

differences in activity ($U=14787.0$, $p=0.001$), with higher activity displayed under favourable weather conditions (mean rank of 1=281.31; 2=178.52). There was no significant population-by-weather interaction (Wald $\chi_{(1)}=1.869$, $p=0.393$).

	Initial model AICC, BIC	Final model AICC, BIC	Population	Temperature	Population*Temperature
<i>Migratory activity</i>	1574.44; 1596.80	1521.76; 1582.0	<0.001	ns	ns

	AICC, BIC	AICC, BIC	Population	Weather	Population*Weather
<i>Migratory activity</i>	1581.04; 1603.53	1578.76; 1593.81	<0.001	<0.001	ns

Table 5: Table of AICC (corrected Akaike information criterion) and BIC (Bayesian information criterion) values as analysis selection criteria for the final GLIM model (Omnibus test $p<0.05$) and p values for each of the factors included in the model. Significant effects are stated by its p value, ns stands for a non-significant effect.

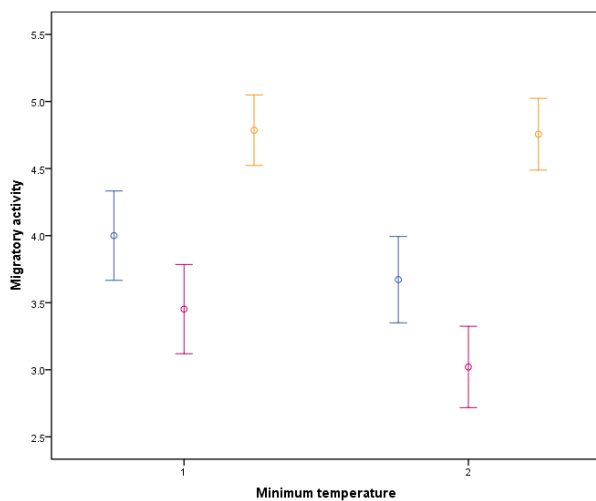


Figure 14: Mean and standard error of the amount of migratory activity in the three populations of blackcaps kept inside aviaries for the two categories nights defined by minimum temperature (1=minimum temperature mean below $<8^{\circ}\text{C}$; 2=minimum temperature $>8^{\circ}\text{C}$); Madrid-blue, Cocentaina-purple, Tarifa-orange

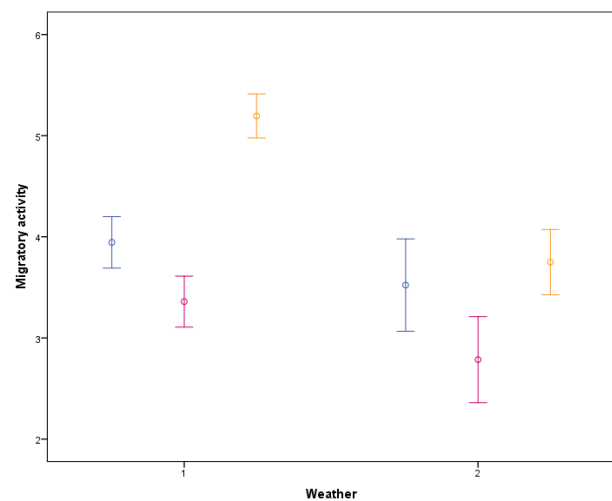


Figure 15: Mean and standard error of the amount of migratory activity of the three populations inside aviaries for the two categories of the weather conditions over the observed period (1-favourable weather with clear skies, 2-unfavourable weather); Madrid-blue, Cocentaina-purple, Tarifa-orange

9.4 Discussion

The main aim of this work was to study the potential effect of environmental variables on migratory activity of individuals housed within two distinct conditions, cages and aviaries; to test the “environmental threshold model of migration” (Pulido, 2011) under semi-natural conditions. In addition, we aimed at investigating the reliability of migratory activity data obtained in aviaries, an environment that allows birds to develop a more natural behaviour, i.e. where they were able to fly in captivity. We found that keeping birds in aviaries had only small effects on the expression of their migratory behaviour. However, Iberian blackcaps



started migratory activity significantly earlier in aviaries than in cages. The mean amount of migratory activity, however, did not differ; only birds from Tarifa tended to be more active in aviaries than in cages.

Weather and its different aspects are considered as the most critical proximal cues for the onset of migratory activity, where different authors consider different features to be the most important (Rappole, 2013), such as barometric pressure (Bagg *et al.*, 1950), temperature (Lack, 1960), direction of winds (Richardson, 1978) or cloud cover (Alerstam, 1978). The differences in migratory activity found between birds kept under two different conditions presumably reflect their exposure to different environmental factors. In our study we found that the migratory activity of individuals kept in aviaries is affected by weather conditions, where bad weather causes a reduction of migratory activity. One of the predictions of the environmental threshold model is that sedentary populations close to the threshold and partial migrants should show more plasticity in the expression of behaviour and a stronger response to specific temperature and weather conditions (Pulido, 2011). In accordance with this predictions, blackcaps from the sedentary Tarifa population demonstrated higher plasticity as reflected by a tendency of being more active inside aviaries than in cages under the direct influence of environmental factors that could have shifted the threshold towards more migratory phenology. We did not find a difference in migratory activity in birds from Cocentaina, probably due to the small sample size (caged birds $N=3$, aviaries $N=4$). On the other hand, the environmental threshold model predicts that the behaviour of migrants in different environments (e.g. cages, aviaries or the wild) should be similar as they find themselves away from the migration threshold and, therefore, the expression of their migratory behaviour is supposed to be more canalized (Pulido, 2011). In accordance with this prediction, we found that the Madrid population showed the smallest behavioural differences between the two experimental conditions. As illustrated in the Figure 1, the migration threshold of each populations depends on the continuous environmental variable E_1 , E_2 and E_3 , where E_1 could be representing the conditions inside cages, at which we cannot distinguish different migratory strategies of the three populations, as it appears that the expression of migratory behaviour is induced in all birds by this particular environment. By testing birds inside aviaries, our idea was to try to expose birds to environmental conditions more to the right of the environmental gradient that determines the migration threshold. Under these conditions we expected that different migratory phenology would be displayed by individuals from the different populations, and to be able to find the environmental variable responsible for that distinction. The results of our experiments showed that in aviaries too all birds from the three populations displayed migratory activity, which indicates that the environmental conditions given in aviaries did not cause a change in the migration threshold. The fact that in aviaries (the amount of) migratory behaviour did not change compared to cages may indicate that with our experiment we did not modify the crucial environmental factors that induce or suppress migratory behaviour. These factors, which were identical between cages and aviaries, were environmental variables associated with geographical latitude (e.g. photoperiod, magnetic field, etc.) or air pressure. These environmental factors we did not control for but may be decisive in determining whether a bird close to the migration threshold will migrate or not. However, we have managed to induce the slight



change in Tarifa population inside aviaries towards tendency of higher migratory activity within the more “natural” (migrant) environment. The shift of the threshold was not sufficient to observe different migratory strategies between populations, however, it did affect the apparently more plastic Tarifa population, confirming the predictions of the “threshold model”.

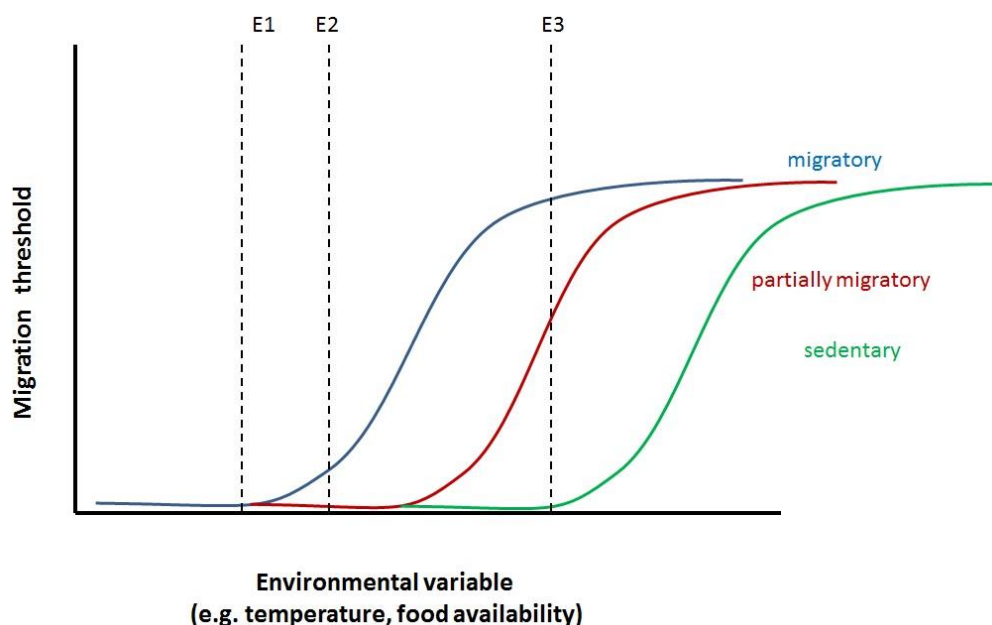


Figure 16: “Threshold model” with three different reaction norms (migratory, partially migratory and sedentary). Environmental conditions (E1, E2 and E3) , such as food availability, temperature etc., determine the migration threshold and, as a consequence, the proportion of migrants in each population (after Pulido, 2011 and Pulido *et al.*, unpublished).

Nocturnal locomotory activity does not always reflect the urge to migrate, as it was interpreted by some authors as an atavistic trait, expressed at a lower level than within natural populations, (Mewaldt *et al.*, 1968; Smith *et al.*, 1969), or it was associated to other types of behaviour, as juvenile or nocturnal dispersal, nomadism or territory acquisition and maintenance (Berthold, 1988; Mukhin *et al.*, 2005; Mukhin *et al.*, 2009). For that reason we have described the predominant types of behaviour within cages and aviaries, to correlate them to the previously described characteristic migratory behaviour in captivity (wing whirring, “beak-up flight”) Gwinner and Czelschlik, 1978; Berthold and Querner, 1988; Berthold *et al.*, 2000; Agatsuma and Ramenofsky, 2006; Coverdill *et al.*, 2011) and discard the possibility that the observed locomotory activity may not reflect migratory activity. In our study we demonstrate that birds inside cages were predominantly displaying wing whirring and that in aviaries flying was the predominant behaviour in all three populations. Judging by the observed behaviour, we could confirm that the recorded nocturnal activity in cages (i.e. predominantly wing whirring) was indeed a display of migratory behaviour (i.e. nocturnal flight), not just escape behaviour or a locomotory activity due to possible disturbances by neighbours or other external factors.

Specifically, we show that the expression of wing-whirring activity in cages could correspond to the flying behaviour of the individuals kept in aviaries. The amount and patterns of night activity differed between



the two environments only within the second period of the observed migration season, and only during the first few hours of the night time, when birds in aviaries were more active. However, the general patterns of night activity did not differ in amount or shape of the night activity distribution. We showed that there are no population or environment specific pattern of migratory activity. Although the main type of behaviour shown by blackcaps in these two environments differ, the patterns and displayed amounts are similar, and the difference in type is probably only a consequence of different housing conditions. As the caged birds are confined to a much smaller place limiting their movement they cannot express their “natural” flying behaviour during migration. Consequently, this “urge to migrate” during the migration seasons becomes jumping around and wing whirring in cages. This has been previously described as “wandering by wing whirring in a sitting position” or “flying with the brakes on” (Berthold and Querner, 1988; Berthold 2000). These findings are leading us to conclude that the method of using aviaries housed birds for quantifying and describing the migratory activity is valid and is accurately showing migratory behaviour under more “natural” conditions. This has been previously undescribed.

We believe that the absence of population differences is a result of the environmental influence as all three populations were housed at the “migratory” Madrid location, responding to its particular conditions. We could conclude that the method of observing migratory activity within cages in captivity has been proved valid, as well as the one observing birds inside aviaries, and that the nocturnal activity displayed in captivity during the migration season is truly a display of natural migratory activity, the type of activity depending on the available space-aviaries provide enough room for flying behaviour, while in cages the only possibility is wing whirring while sitting on the perch or jumping.

The critiques of the reliability of recorded migratory activity inside cages usually refer to the experimental setup as birds were usually kept in artificial conditions that could affect the migratory behaviour. In order to understand these possible alterations, a comparison with free-flying birds is necessary (Rappole, 2013). As our results demonstrate that the observed nocturnal activity was truly migratory activity and that is highly comparable to the migratory activity of the birds within aviaries, a more “natural” environment where they were able to fly, these results could serve for the interpretation of previous studies conducted in cages and confirm their reliability.

Future experiments on birds, testing populations of different phenotypes by environmental conditions, are necessary to additionally confirm the “extended threshold model”, a model useful for studying real migration movements as it includes the environment as an important factor in the determination of the migratory phenology (Olsson *et al.*, 2006; Brodersen *et al.* 2008, Skov *et al.*, 2010; Chapman *et al.*, 2011; Chapman *et al.*, 2012; Pulido, 2011).

Our results can be considered as very promising for opening a research agenda for testing predictions about migratory behaviour and the effect of environmental canalization, where expression of migration is insensitive to environmental variation, by using populations on a bigger geographical scale; birds with



different tendencies to migrate, e.g. exposing northern migratory populations to conditions characteristic for a sedentary population, but in an experimental setup similar to natural conditions, like outside aviaries. The results could be later on used for prediction of the populations' evolutionary changes in migration under changing environment.



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9.6 Supplementary material

	Onset		Amount
	PC	PC1	PC
% of variance	76.22	75.53	96.36
Eigenvalue	3.049	1.511	3.855
Factor loadings			
Onset of MA 5 days criteria	0.850	0.869	
Onset of MA 5 days criteria higher activity	0.904		
Onset >3 intervals of activity in the central part of the night	0.915	0.869	
Onset >4 intervals of higher activity in the central part of the night	0.819		
Average MA corrected for the maximum number of days of activity			0.991
Sum of 30min activity corrected for days with more than >3 intervals and missing data			0.946
Sum of 30min activity for the entire activity period			0.992
Sum of 30min activity for the entire activity period corrected for the days with 1 and 2 30min activity sums			0.997

Table 1: Factor loadings and % of explained variance for the principal component factor analysis of the onset of migratory activity and amount of the migratory activity for the autumn season; PC –PC onset; PC1 – PC onset act1; PC-PC amount

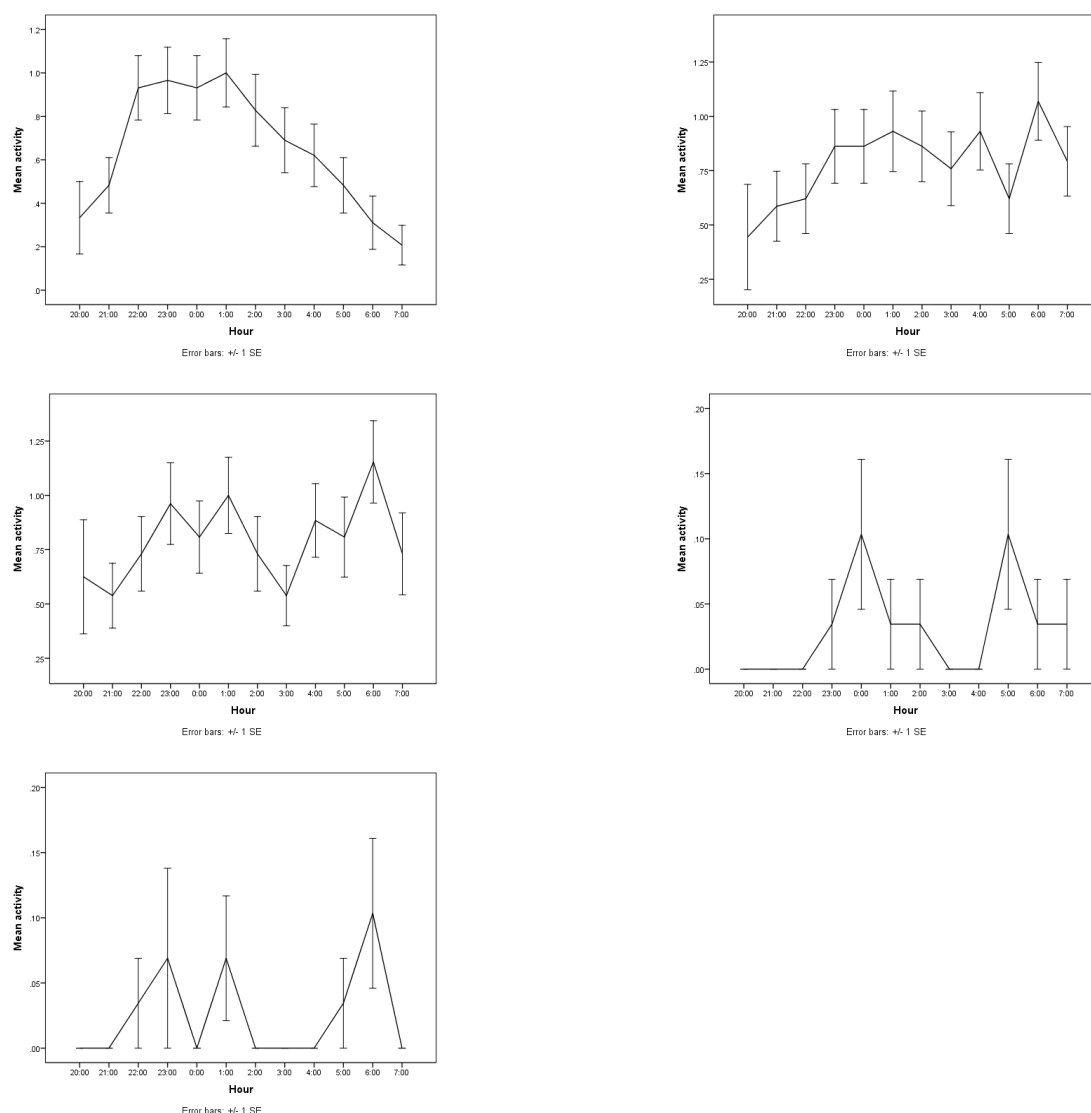


Figure 1: Mean values of half hour sums during the night time over the September–November period for the Madrid individuals housed inside cages

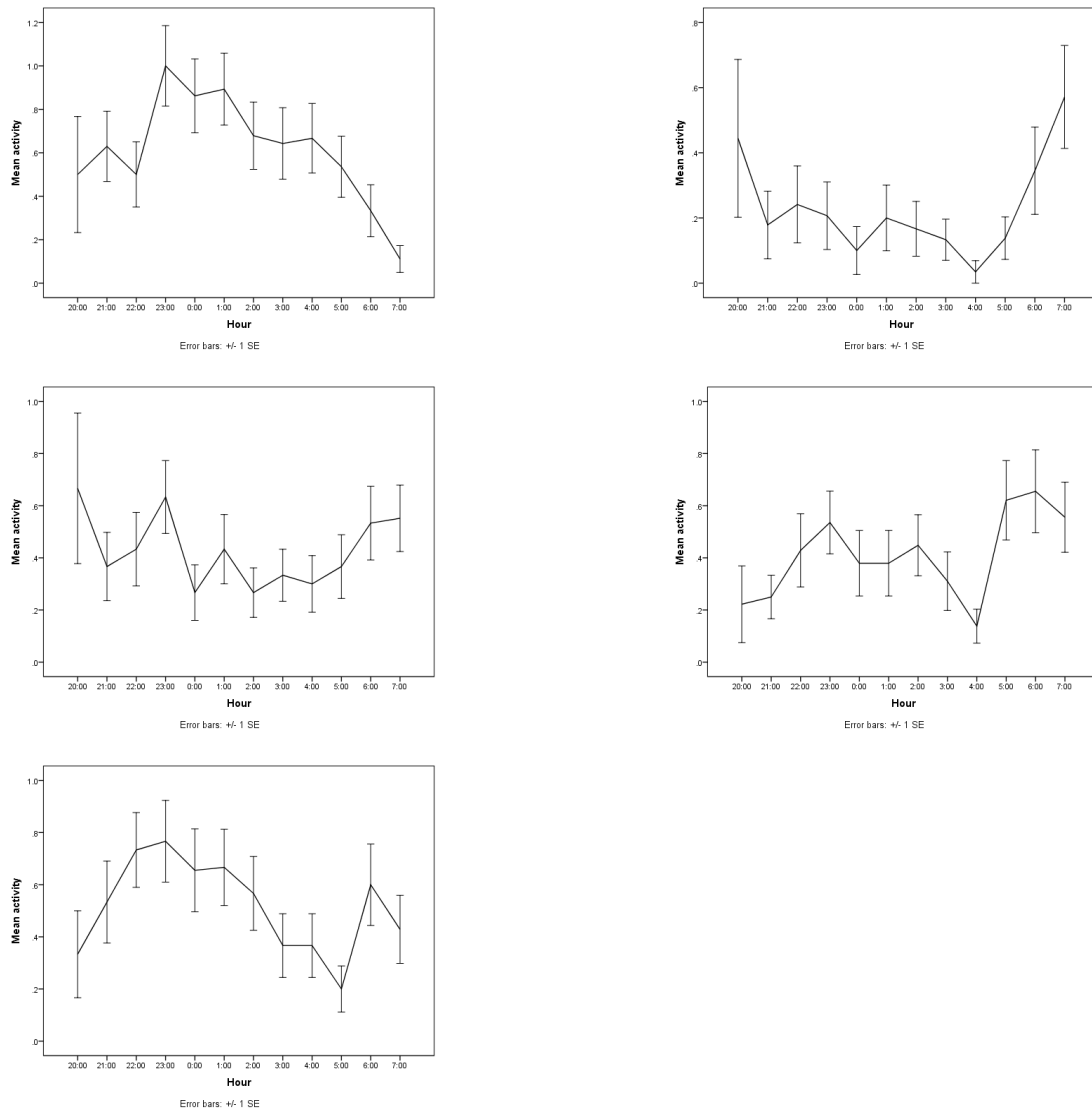


Figure 2: Mean values of half hour sums during the night time over the September-November period for the Madrid individuals housed inside aviaries

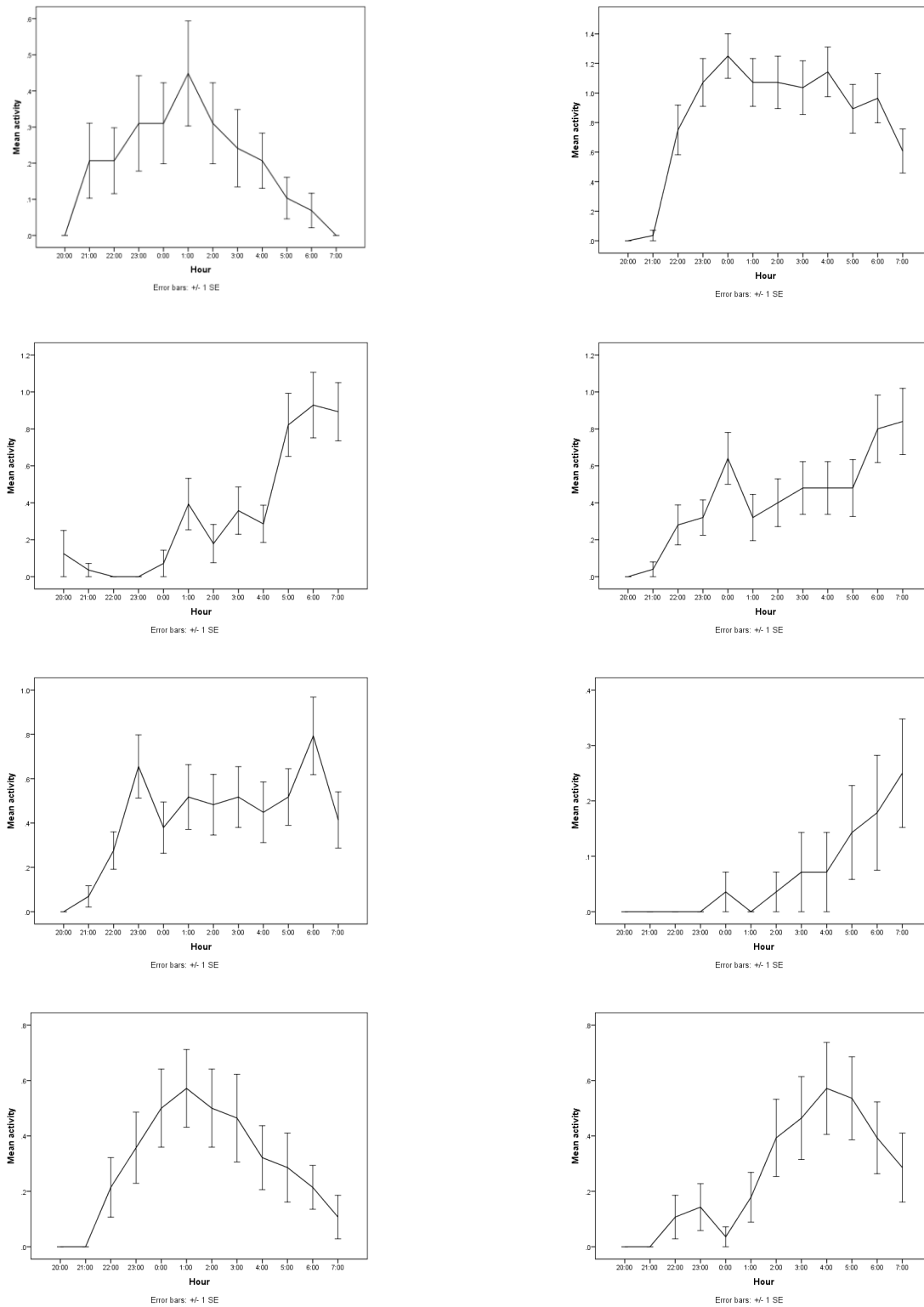


Figure 3: Mean values of half hour sums during the night time over the September-November period for the Coccyzus erythrophthalmus individuals housed inside cages

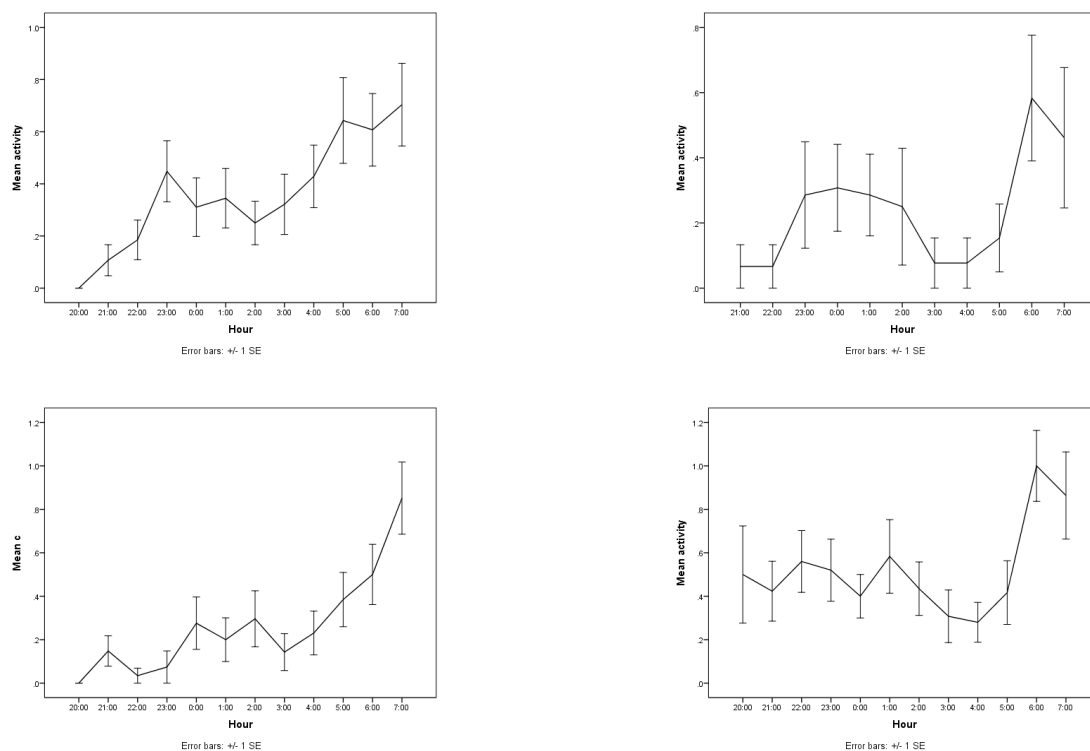
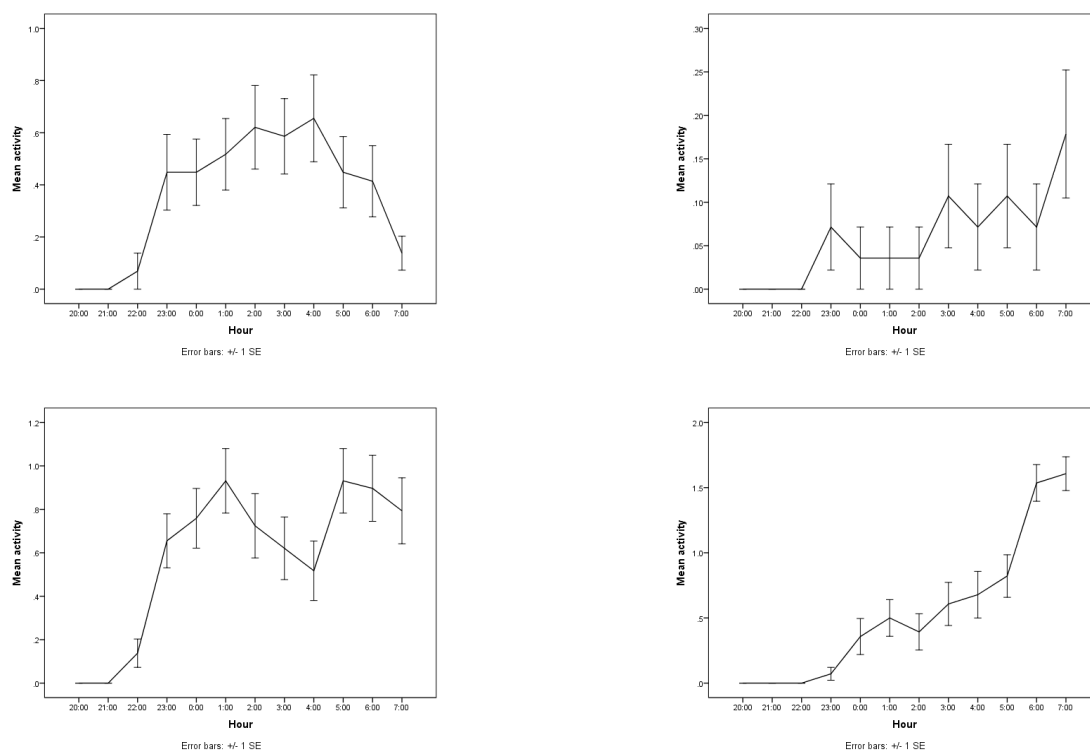


Figure 4: Mean values of half hour sums during the night time over the September-November period for the Cocentaina individuals housed inside aviaries



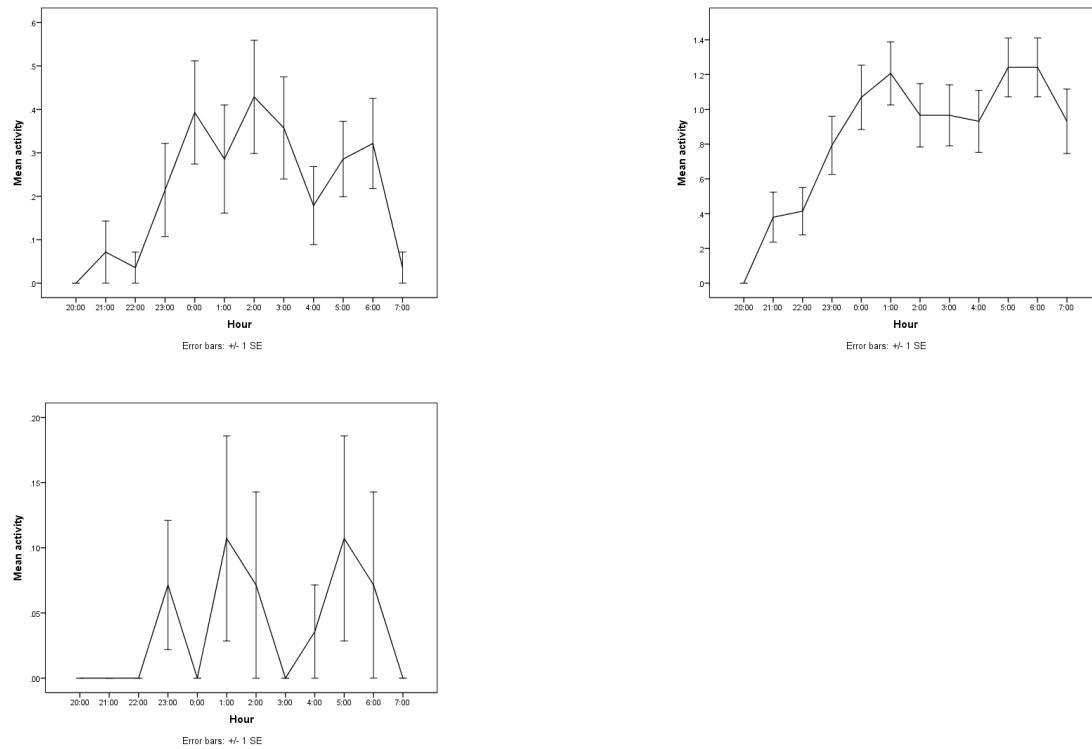
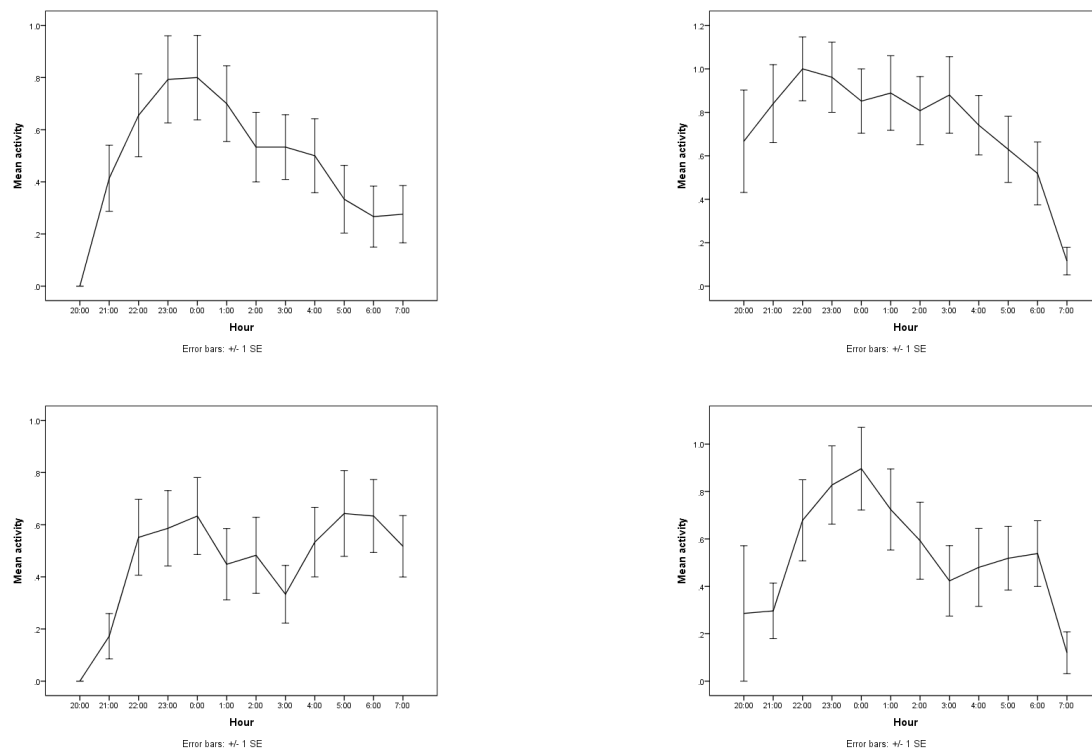


Figure 5: Mean values of half hour sums during the night time over the September-November period for the Tarifa individuals housed inside cages



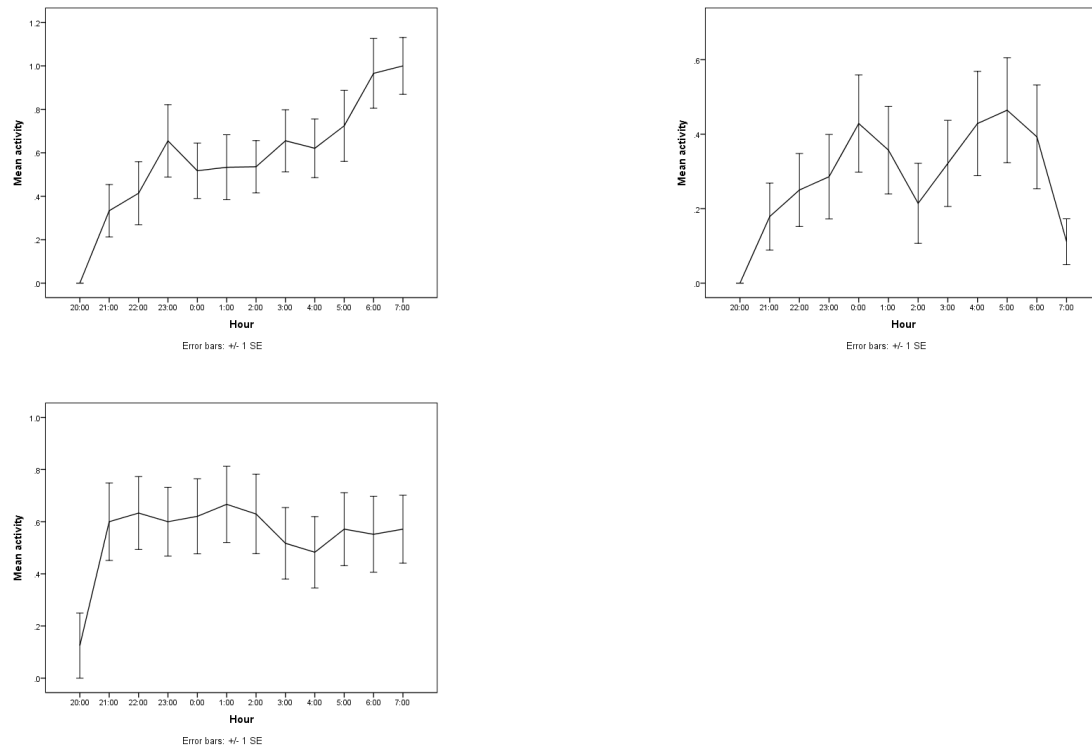


Figure 6: Mean values of half hour sums during the night time over the September-November period for the Tarifa individuals housed inside aviaries





10 Chapter 3

**“Are exploration and neophobia associated with migratory
behaviour? An experimental study in blackcap
(*Sylvia atricapilla*)”**

Bulaic M, Gallardo M, Pulido F (unpubl. manuscript)





10.1 Introduction

The reaction of an individual can differ when faced with unfamiliar situations. It has been demonstrated in many species that this response of an individual to mild stress is similar across situations, but differs among individuals (Sih *et al.*, 2004b). Such consistent differences in behaviour have been termed “animal personalities” (Gosling, 2001; Réale *et al.*, 2007) or, when referring to a suite of correlated behaviours, “behavioural syndromes”, (Sih *et al.*, 2004a; 2004b). They reflect difference in behaviour when coping with new information, by developing different behavioural (Sih *et al.*, 2004b; Carere *et al.*, 2005; Bell *et al.*, 2009) and physiological strategies (Carere *et al.*, 2001; Carere *et al.*, 2003b, Carere and van Oers, 2004). One characteristic of these strategies is that they are maintained over time and situations (Sloan Wilson *et al.*, 1994; Sih *et al.*, 2004b; Réale *et al.*, 2007), in some cases even between natural and experimental conditions (Herborn *et al.*, 2010). This constancy is usually measured by its repeatability (Lessells and Boag, 1987). Aside from being repeatable, “animal personalities” are characterized by being heritable, having a moderate genetic component (Dingemanse *et al.*, 2002; van Oers *et al.*, 2004; van Oers and Mueller, 2010).

“Personality” types can be classified along major axes, usually varying continuously between two extremes, rather than having a bimodal distribution. For example, some individuals could behave in certain situations more aggressively, be more active or bold, while others generally are less aggressive, passive or shy. The most notable axes of animal “personalities” are (1) aggression, where an individual could be aggressive or passive (Huntingford, 1976); (2) activity, with active (proactive) and inactive (reactive) individuals (Sih, 1992; Hessing *et al.*, 1994; Koolhaas *et al.*, 1999); (3) exploratory behaviour differentiating fast and slow explorers (Verbeek *et al.*, 1994), and (4) risk responsiveness of an individual, varying along an axis of neophobia-neophilia, being shier or bolder (Sloan Wilson *et al.*, 1994; van Oers *et al.*, 2004) and sociability with more or less social individuals (Cote *et al.*, 2010).

Behaviours can be beneficial under certain environmental conditions, while in other situations, they may be disadvantageous. These trade-offs may maintain variation in personality types. For instance, some individuals bold in exploration of novel objects, or in reaction to predators, could undertake higher risks, which under conditions of high predation could result in increased mortality (Sih *et al.*, 2012). Hence, various personality types have different predispositions to adjust and respond to changing environmental conditions (Koolhaas *et al.*, 1999; Coppens *et al.*, 2010). For example, “slow” explorers pay close attention to changes in their environment, which has a selective advantage in moderately variable environments (Carere, 2003b; Sih *et al.*, 2004a; van Overveld and Matthysen, 2013; Niemela *et al.*, 2013). On the other hand, proactive individuals, or “fast” explorers (= “bold” personalities), investigate the environment more quickly, tend to be more aggressive, are less innovative and more prone to routine-like behaviours in their responses, probably requiring more time to change the routine accordingly as they are driven by previous experiences (Verbeek *et al.*, 1994; Verbeek *et al.*, 1996). As a consequence, “bold” individuals have an advantage in relatively



constant or highly variable environments where flexibility is not advantageous (Sih *et al.*, 2004a; Niemela *et al.*, 2013).

Aside from different personality types being adaptive in different environments, different life styles can influence the fitness of personality traits as well (Mettke-Hofmann *et al.*, 2002; Mettke-Hofmann *et al.*, 2005b; Mettke-Hofmann *et al.*, 2009). For instance, individuals with different migratory life styles (i.e. migrants and residents) are exposed to new environmental conditions differently. During their lives, migratory and sedentary populations face various challenges that require appropriate responses. Migrants are confronted with unfamiliar habitats and stay in various areas for a relatively short periods of time. In contrast, residents remain in the same area year-round, and have to cope with seasonal changes, where extensive knowledge of their environment is likely to be highly beneficial (Mettke-Hofmann *et al.*, 2005b). To date there are only few studies that have tested this association and their results have been contradictory. For example, in a study comparing exploratory behaviour in resident Sardinian warblers (*Sylvia melanocephala*) and migratory garden warblers (*Sylvia borin*), Sardinian warblers resulted to be less neophobic and had longer exploration times of a novel object within their familiar cage (Mettke-Hofmann *et al.*, 2005a; Mettke-Hofmann *et al.*, 2005b; Mettke-Hofmann, 2007). These results support the hypothesis that sedentary birds need to gain a detailed knowledge of their environment. In another study on 10 parrot species it was demonstrated that resident species approached novel objects faster than migratory species (Mettke-Hofmann *et al.*, 2005a). However, when the two warbler species, Sardinian and garden, were under a novel environment experiment, the garden warblers showed to be less neophobic and fast explorers (Mettke-Hofmann *et al.*, 2009). Aside from the exploratory behaviour, other axes of personality, like risk responsiveness, have been shown to be related to migratory strategy. For instance, boldness increased the probability of developing migratory activity in roach (*Rutilus rutilus*) (Chapman *et al.*, 2011).

As we can see from the literature, previous studies aiming at elucidating the relation between avian personalities and migration have obtained contradictory results. Moreover, most studies tested this association between migratory and exploratory behaviour by comparing species. The only intraspecific study was conducted in the blue tit, a facultative migrant that shows little, partly irregular migratory movements, with however, opposite results to previously stated studies; migratory individuals had shorter latencies in approaching the novel object than residents (Nilsson *et al.*, 2010). Thus, in view of the contradictory results and limitations of previous studies, we aimed at exploring the correlation of different aspects of “personality with individual migratory activity within and among populations of one species. For this study we selected populations with different migratory strategies, the two extremes, i.e. a fully migratory and completely resident population, and a partially migratory population. In the partially migratory population, we expected to find large variation in migratory behaviour, and migration control to be more flexible and more likely to be influenced by environmental variation (Pulido, 2011).



10.2 Materials and methods

10.2.1 Subjects of the study

For our study, we used juvenile blackcaps (*Sylvia atricapilla*), a common and widespread Passerine species that has previously used as a model for the study of the control and evolution of migration (Berthold, 2001; Pulido, 2007). Birds were captured in 3 Iberian populations: Madrid (Pinilla del Valle 40°55'N, 3°49'W), Cocentaina (Alicante) (38°44'N, 0°26'W) and Tarifa (Los Barrios 36°11'N, 5°36'W). We selected these populations because they allowed us to study differences in personality regarding their migratory strategy on a small geographical scale. Based on observations and ringing recoveries, blackcaps from the Madrid population are considered migratory (Tellería *et al.*, 2001), birds from Cocentaina partially migratory (Morganti *et al.*, 2015) and the Tarifa population sedentary (Tellería *et al.*, 2001). Juveniles were captured in summer, between the end of June and the beginning of August, in 2010 (N=33), 2011 (N=33) and 2012 (N=22). Each bird was banded with a colour ring so that it could be easily identified throughout the experiment. After capture, birds were transported to our facilities in the large restricted area within Madrid (natural park Casa de Campo, 1723 ha, 40°25'N, 3°45'W), wooded with holm oaks (*Quercus ilex*) and typical Mediterranean vegetation. Birds were kept in individual cages (45x23x38 cm) with two movable perches, feeder and two drinkers within an unheated shed (5x2m) in a secluded part of the park, under temperature with $\pm 4^{\circ}\text{C}$ difference to the outdoor temperature conditions. Birds indoors received natural daylight entering through two windows. To reach outdoor light intensity, the room was illuminated with two additional lights (2 compact fluorescent lamps, Megaman WL 130 Compact 2000 HPF, with a power of 30 W, a light intensity of 1620 lumen and a colour temperature of 6500 K=daylight), which were adjusted to the natural photoperiod weekly, mimicking natural conditions (weather station at Barajas, Madrid; http://www.tutiempo.net/tiempo/Madrid_Barajas/LEMD.htm). As migratory activity is not expressed in complete darkness (Helms, 1963) a night light (2-3 Lux) was mounted centrally in the experimental chamber, which was switched on throughout the experiment.

Aviaries (3x2x2.3m) were equipped with 6 perches in three corners, two feeders, two water plates, a natural undergrowth and overhanging holm oaks, exposed to the natural photoperiod and temperature fluctuations (range: -1°C - 40°C in the sun). All individuals had both food and water *ad libitum* daily replenished (fresh fruit of the season, mealworms, industrial food for insectivorous birds “Raff” with addition of vitamins). Within the shed they had visual contact with individuals in neighbouring cages, and auditory contact with all individuals, while in aviaries only auditory contact was possible. After the experimental period was finished, at the end of May of each experimental year all individuals were released after removal of colour rings at the exact sites where they had been captured originally.



10.2.2 Experimental design

All birds were kept under same conditions, in a so-called “common garden”, which potentially allows determining genetic differences in personality among our three populations. Experiments were conducted both in autumn and spring during the naturally occurring migration periods. In all three experimental years this autumn migration period was from the first week of October until the first week of November. Spring migration period was from the last week of February through the end of March. The exact migratory period was determined by measuring “Zugunruhe”, i.e. nocturnal migratory activity, as described in Coppack and Pulido (Coppack and Pulido, 2004). Behavioural experiments were filmed with 2 digital cameras (Panasonic SDR-H85 and Sony DRC-SX65E) for 20 minutes. During all experiments the observer was not present in the test room. All the experiments within one migratory season were repeated 3 times within 2-3 days. Hence, each experiment was repeated 6 times in each individual. As birds were able to see each other in the exploration experiments, we accounted for it when analysing the data, to test for a possible effects of copying.

10.2.2.1 Moderate stress experiment

The aim of this experiment was to determine if differences exist between our 3 populations in their response to a moderately stressful situation. This experiment is a modification of the protocol we usually used for feeding birds, yet it included a short period of food deprivation. The observer, whose presence the birds were already familiar with, would remove the usual feeder for some 20-30 min, to deprive the individuals of food, and after that period, the original feeder with newly replenished food would be returned. The variables of interest were the amount of time, after returning the feeder, each individual bird needed to approach the usual feeder, and to feed for the first time (i.e. latency). Other variables measured were the total time spent in the feeder and the number of times that individuals approached the feeder (number of visits), both before feeding for the first time.

10.2.2.2 Exploration test

In the exploration test, after 20-30 min of food deprivation, a new type of feeder (clay dish with 2cm high edges) filled with food was introduced into the cage and placed on the floor of each cage, while the usual feeder, which was placed back on the cage at the same time, remained empty. With this experiment, we tested for differences in the way individuals adjust their behaviour to this “altered” environment. We were interested in determining how fast birds would explore the “new food source” and how fast they would change their routines. Measured variables were the same as in the moderate stress experiment; latency to approach the usual feeder, latency to feed, number of visits to the usual feeder and time spent on it prior to feeding; with the addition of three new variables: latency to approach the new feeder, latency to feed from the new feeder for the first time, and number of visits to the new feeder.

10.2.2.3 Memory test

The design of this experiment was the same as the previous one, except that, here, the new feeder was covered with a piece of white paper so the food would not be visible to the bird. As the experiment was repeated for 3 times, it is to be expected that birds have accustomed to the new food location, although this time the food was hidden. Variables tested are the same as in the exploration test.

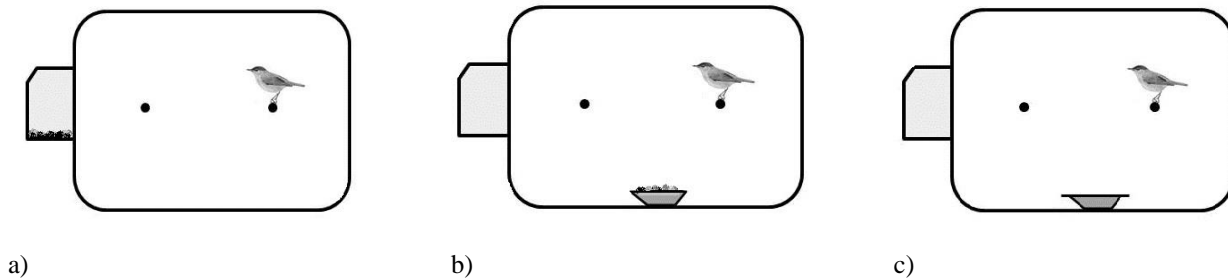


Figure 1: Illustrations of the experimental setups; a) moderate stress experiment with the usual feeder filled with food placed onto the cage after 20-30min food deprivation; b) exploration test, empty usual feeder placed at the same time as the new feeder, filled with food, at the cage floor; c) memory test, with the same setup as the exploration test, only the newly added feeder at the cage floor is now covered with a piece of paper to hide the food from the direct sight.

10.2.3 Migratory activity

Nocturnal activity, restlessness or “Zugunruhe”, is a good indicator of migratory activity in birds in captivity (Berthold, 1996), displayed by wing whirring and flapping and jumps (Berthold *et al.*, 2000). To determine migratory activity of individuals, nocturnal activity was recorded using movable perches with micro-switches and a Microscript recorder (see details in Chapter 1). It was measured in 30-minute intervals during the lights out period for the individuals inside the cages that were connected to the recording system (for reference see Chapter 1).

10.2.4 Statistical analysis

For the statistical analysis, IBM’s SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp) was used. The goal was to test difference among populations in the latency to feed in each of the three experimental setups. The dependent variables analysed are the following: latency to feed, latency to approach the usual/new/covered feeder, total time spent in/around the usual/new/covered feeder and number of visits to the usual/new/covered feeder. Once the bird would feed, the experiment for this individual was considered as finished. Only the first of each experiment was used for the effects analysis. Individuals that failed to conclude the task (i.e. which did not feed within 20 min) were excluded from the analysis because of uncertainty of the time when those birds would fulfil the task. As in the analysis of the exploration and memory test many individuals had to be excluded for this reason, which strongly affected the power of the analyses, we transformed latencies into a dichotomous variable (0 for not approaching/feeding and 1 for doing so within 20 minutes). We analysed this dichotomous variable using contingency tables.



We conducted principal component analyses (PCA) separately for each experiment on all of its extracted variables in order to obtain one variable that includes different personality axes and defines better the differences in personalities. One principal component (PC) was extracted for the moderate stress experiment using the latency variables in the first test (latency to feed, to approach the usual feeder and total time spent in the usual feeder before feeding; number of visits was excluded due to low number of data). For both the exploration and memory test, three components were extracted: PC1 from the latency to feed, latency to approach the new feeder and time spent inside usual feeder; PC2 from number of visits to the new feeder and total time spent around the new feeder before feeding; PC3 from latency to approach the usual feeder, total time spent in the usual feeder and number of visits to the usual feeder, as well, prior to feeding (see Table 1 for details). The original variables and PC's were analysed by fitting Generalized Linear Interactive Modelling (GLIM) models. For the selection of models we used a stepwise backward approach, basing the selection of variable on the corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC). Latency to feed and to approach the new/covered feeder in the exploration and memory test were not normally distributed, even after trying different normality transformations. Therefore, these variables were not tested for GLIM models.

As the effect of sex was not significant in any of the variables, it was not included in the final analyses and, therefore, not presented in the results. Repeatability of individual feeding latency was calculated on Z transformed variables following the formula proposed in Lessells and Boag (Lessells and Boag, 1987) through one way ANOVA. For estimating repeatability of performance in the exploration and memory tests, we used only the data from the 2011 cohort, as these were the only birds tested in two subsequent seasons.

All birds showed nocturnal migratory restlessness. Two types of activity were determined based on the displayed amount of activity during the 30min period as a reference point; lower activity where the birds were active for at least half the 30min period, higher activity with birds active for the most of the 30min period.

The onset of migratory activity (MA) was defined for each individual as the first Julian date of a period with continuous activity at which the sum of activity per night was equal or higher than five 30 min intervals during at least five consecutive days, ("5-day onset of MA" criterion) (see Pulido *et al.*, 2001; Coppack *et al.*, 2001; Pulido and Coppack, 2004). Similar criteria were applied on extraction of the rest of the variables (see Chapter 1; Table 1 for details and summarized description of the variables).

Principal component analysis for the onset of migratory activity included all the five created variables (PC onset, 74.7% of variance). A second principal component was created using only the onset of the lower activity (PC onset act1 80.2%), and third of the higher activity (PC onset act2, 93.4%).

The amount of the migratory activity (MA) was measured as the sum of 30min periods of night activity during the migration season, which was defined as the period between the onset and the end of migratory

activity, for lower and higher activity, corrected for the number of days with missing data or low activity (see Chapter 1 for detailed definitions).

PC analysis for the amount of migratory activity was done with all created variables for both lower and higher levels of activity where two components were extracted, PC amount of activity (67.7% of variance) and PC amount of the higher activity (act2, 23.1%) (loadings in Supplementary table 1).

10.3 Results

The extracted principal components for the three experiments reflect different personality axes (Table 1). The principal component “moderate stress” represents the bird’s stress response, being primarily determined by latencies. Both in the exploration and the moderate stress experiment, we extracted three principal components: PC1 could be interpreted as the general stress response on the presented task within the experiment, where we see both high latency for approaching the new as well as the usual feeder, suggesting that depending on the animal’s personality it takes the bird longer or shorter time in general to perform any kind of action. PC2 of both experiments could be interpreted as the neophobic/exploratory axis, as deduced from the high loadings of the number of visits to the new or covered feeder and longer time spent exploring it. PC3 has as similar factor loadings of variables in both the exploration and memory test. Here we have a high positive loading for the latency to approach the old feeder and a high negative loading for the number of visits and total time spent in it. This axis could represent the ability (or inability) of changing habits and to learn or adjust to the new situation. Birds with low PC3 values stick to the old habit, approach to the old feeder faster and spend more time in it looking for the food, where there is none.

a)	Moderate stress experiment	PC		
	% of variance	74.383		
	Eigenvalue	2.232		
	KMO measure of sampling adequacy	0.533		
	Bartlett's test of sphericity	<0.001		
	Factor loadings			
	Latency to feed	0.961		
	Latency to approach the usual feeder	0.914		
	Total time spend around the usual feeder	0.686		
b)	Exploration test	PC1	PC2	PC3
	% of variance	45.564	19.226	17.841
	Eigenvalue	3.189	1.346	1.249
	KMO measure of sampling adequacy	0.558	0.558	0.558
	Bartlett's test of sphericity	<0.001	<0.001	<0.001
	Factor loadings			
	Latency to feed	0.965	0.002	0.191
	Latency to approach the new plate	0.963	-0.076	0.144
	Total time spend around the usual feeder	0.718	-0.123	-0.515
	Number of visits to the usual feeder	0.649	0.168	-0.609
	Latency to approach the usual feeder	0.603	-0.003	0.742
	Number of visits to the new feeder	0.154	0.802	0.003
	Total time spent around the new feeder	-0.088	0.808	0.062



c)	Memory test	PC1	PC2	PC3
	% of variance	50.965	18.074	14.48
	Eigenvalue	3.568	1.265	1.014
	KMO measure of sampling adequacy	0.656	0.656	0.656
	Bartlett's test of sphericity	<0.001	<0.001	<0.001
	Factor loadings			
	<i>Latency to feed</i>	0.968	0.087	0.087
	<i>Latency to approach the covered plate</i>	0.967	-0.009	0.102
	<i>Total time spend around the usual feeder</i>	0.914	0.066	-0.300
	<i>Number of visits to the usual feeder</i>	0.790	-0.238	-0.393
	<i>Latency to approach the usual feeder</i>	0.484	0.033	0.828
	<i>Number of visits to the covered feeder</i>	0.037	0.777	0.096
	<i>Total time spent around the covered feeder</i>	0.009	0.769	-0.237

Table 1: Factor loadings, percentage of explained variance, Keiser-Meyer-Olkin measure of sampling adequacy (KMO; >0.5) and Bartlett's test of sphericity (<0.05), for the principal component factor analysis of a) moderate stress experiment, b) exploration and c) memory tests. Only components with Eigenvalues > 1 are given.

10.3.1 Population effect

10.3.1.1 Moderate stress experiment

The PC moderate stress GLIM model analysis showed a significant population effect (Wald $\chi_{(2)}=20.303$, $p<0.001$) (Supplementary table 2). A one-way ANOVA ($F_{(2,96)}=4.929$, $p=0.009$) confirms the effect where a post-hoc test of pairwise-differences revealed that population differences are result of Madrid's highest and Tarifa's lowest latencies (Tukey's HSD $p=0.006$; Figure 2). The separate analysis of the variables composing the PC1 showed the same among-population differences.

10.3.1.2 Exploration experiment

A significant population effect was found for PC2 (Wald $\chi_{(2)}=6.205$, $p=0.045$) and PC3 (Wald $\chi_{(2)}=18.142$, $p<0.001$) but not for PC1 (Wald $\chi_{(2)}=1.038$, $p=0.595$) (see figures 3,4).

A more detailed analysis of the PC3 demonstrated significant differences in mean latencies ($F_{(2,49)}=9.113$, $p<0.001$) between Cocentaina and Tarifa (Tukey's HSD $p<0.001$) (Figure 3), while Madrid and Tarifa only tended to differ in mean values (Tukey's HSD $p=0.068$). A significant population effect was found as well for the latency to approach to the usual feeder and the total time spent in it (see Supplementary table 2).

10.3.1.3 Memory test

None of the three extracted principal components showed significant population or season effects, neither using the GLIM model analyses nor one-way ANOVAs.

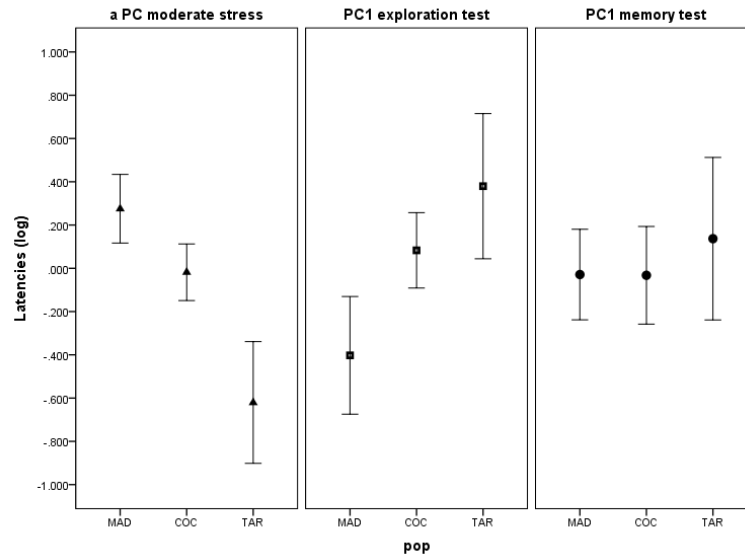


Figure 2: Mean \pm 1SE of the log transformed latency values (s) for the PC moderate stress (triangles), PC1 of the exploration test (squares) and PC1 of the memory test (filled circles), for the three studied populations; MAD – Madrid, COC - Cocentaina and TAR - Tarifa

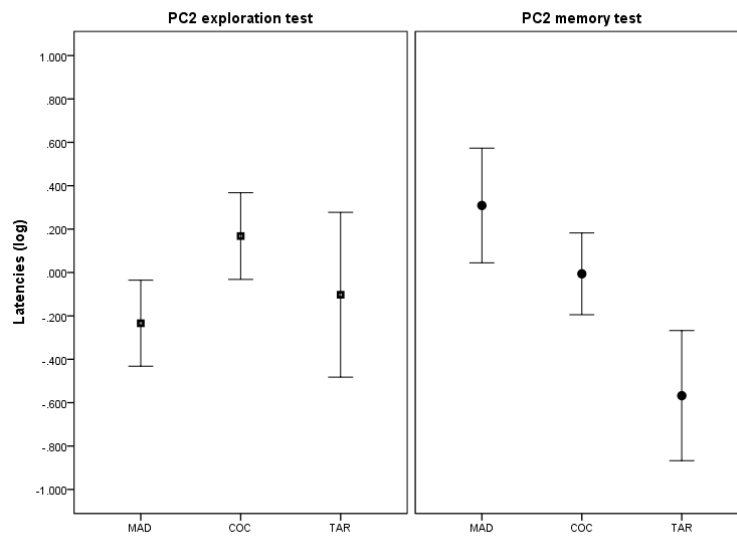


Figure 3: Mean \pm 1SE of the log transformed latency values (s) for the PC2 exploration test (squares) and PC2 of the memory test (circles) for the three studied populations; MAD – Madrid, COC - Cocentaina and TAR - Tarifa

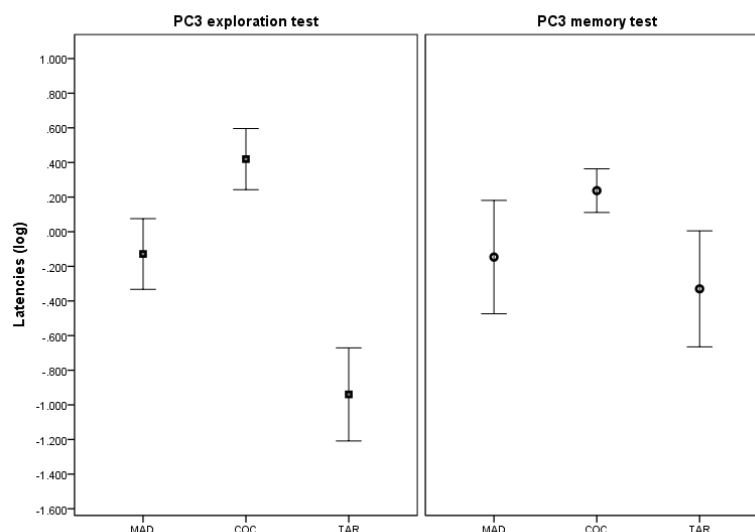


Figure 4: Mean \pm 1SE of the log transformed latency values (s) for the PC3 exploration test (squares) and PC3 of the memory test (circles) for the three studied populations; MAD – Madrid, COC - Cocentaina and TAR - Tarifa

10.3.2 Effect of cohort

Cohort was found to have no significant effect on any of the original variables or the principal component in the moderate stress experiment. However, the PC1 and PC2 of the exploration test did show differences among cohorts (PC1 Wald $\chi_{(3)}=15.199$, $p=0.002$; PC2 Wald $\chi_{(4)}=12.841$, $p=0.012$). The analysis of PC3 showed no cohort effect, but the original variables contributing to the principal components, namely latency to approach the usual feeder (Wald $\chi_{(3)}=13.516$, $p=0.004$) and number of visits to the usual feeder (Wald $\chi_{(4)}=19.695$, $p=0.001$), showed a significant cohort effect.

Regarding the memory test, significant effects were found for the total time spent around the covered feeder (Wald $\chi_{(2)}=26.426$, $p<0.001$) and number of visits to the usual feeder (Wald $\chi_{(2)}=15.018$, $p=0.001$).

10.3.3 Analysis of task “performance” as a categorical variable

10.3.3.1 Exploration test

Looking at the entire duration of the experiment, populations statistically differ in the numbers of feeding/non-feeding individuals within the interval of observation (i.e. 20 minutes) ($\chi_{(2)}=8.297$, $p=0.016$) where Cocentaina had the highest percentage of the individuals that accomplished the assignment (82%), and Madrid the lowest (55%). The percentage of feeding individuals of Tarifa is quite high as well (70%) but statistical differences with the other populations were not found, possibly because of the sample size in this population (Figure 6). There are no statistical differences between autumn and spring within each of the populations, while within season comparison revealed difference in spring ($\chi_{(2)}=9.045$, $p=0.011$) where

Cocentaina has the highest percentage of individuals feeding (87%), Madrid the lowest (50%), and Tarifa (77%) with an intermediate proportion.

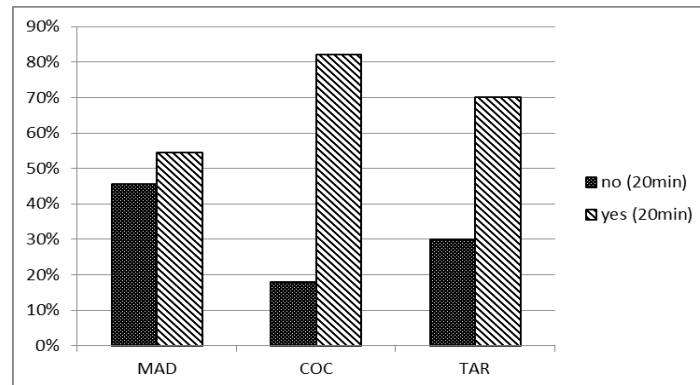


Figure 5: Percentage of individuals finishing successfully the exploration test within 20 minutes, Madrid N=44, Cocentaina N=50, Tarifa N=20; MAD – Madrid, COC - Cocentaina and TAR - Tarifa

10.3.3.2 Memory test

When analysing the proportion of individuals feeding or not within 20 min, only a tendency was found ($\chi^2=5.166$, $p=0.076$) for the populations Madrid (62%), Cocentaina (82%) and Tarifa (81%) to differ. Analysing separately each of the two seasons, populations only tended to statistically differ within autumn ($\chi^2=5.118$, $p=0.077$), where 59% of Madrid, 89% of Cocentaina and 57% of Tarifa individuals finished the experiment within the given time. Here, as well, Madrid ($\chi^2=0.094$, $p=0.759$) and Cocentaina ($\chi^2=1.183$, $p=0.277$) did not differ between seasons, while Tarifa did ($\chi^2=4.747$, $p=0.029$).

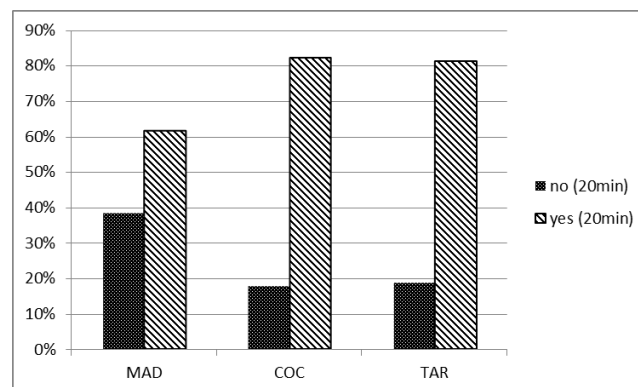


Figure 6: Percentage of individuals finishing successfully the memory test within the time of 20min; Madrid N=39, Cocentaina N=45, Tarifa N=16; MAD – Madrid, COC - Cocentaina and TAR – Tarifa.

10.3.4 Repeatability

Overall repeatability between individuals in feeding latency in the moderate stress experiment was quite high (0.37), with similar values within both Madrid (0.29) and Cocentaina (0.25), with higher values within autumn season compared to spring.



Z transformed latency to feed	Moderate stress			Exploration test			Memory test		
	r	F	p	r	F	p	r	F	p
Overall	0.37	4.784	<0.001	0.44	5.659	<0.001	0.42	5.326	<0.001
Within season									
Autumn	0.49	3.963	0.001	0.54	4.589	<0.001	0.60	5.567	<0.001
Spring	0.32	2.464	0.016	0.62	6.034	<0.001	0.35	2.662	0.002
Among seasons									
Mean season value	0.43	2.561	0.013	0.45	2.679	0.010	0.72	6.398	<0.001
1 st replica of the season	0.14	1.324	0.250	0.22	1.557	0.144	0.32	2.207	0.032
MADRID									
Overall	0.29	2.244	0.024	0.46	6.094	<0.001	0.38	4.723	0.008
Within season									
Autumn	0.52	4.253	0.010	0.49	3.909	0.003	0.52	4.316	0.001
Spring	0.45	3.476	0.021	0.74	9.564	<0.001	0.07	1.254	0.327
Among seasons									
Mean season value	0.50	3.023	0.035	0.44	2.605	0.057	0.62	4.538	0.007
1 st replica of the season	0.27	1.727	0.181	0.16	1.391	0.289	0.26	1.654	0.108
COCENTAINA									
Overall	0.25	1.978	0.047	0.36	4.328	<0.001	0.41	5.205	0.005
Within season									
Autumn	0.50	4.031	0.012	0.62	6.092	<0.001	0.56	4.856	0.001
Spring	0.13	1.442	0.269	0.19	1.727	0.127	0.310	1.975	0.098
Among seasons									
Mean season value	0.38	2.203	0.095	0.43	2.495	0.066	0.83	11.895	0.001
1 st replica of the season	0.00	0.996	0.500	0.26	1.706	0.186	0.49	3.111	0.032

Table 2: Repeatability estimates (r) for the Z transformed latency to feed from the usual/new/covered feeder for the three personality experiments, and calculated by one way ANOVA for the 2011. This cohort was the only one with two consecutive seasons of conducted experiments, containing only Madrid and Cocentaina populations; non-significant repeatability values are in italic.

10.3.5 Behavioural syndrome

As the behavioural syndrome implies a suite of correlated behaviours, the PCs for all of the three personality experiments were tested for possible correlations (Pearson's). The only significant correlations were found between PC moderate stress and PC3 exploration test ($r=0.401$, $p=0.017$, $N=35$), and PC moderate stress and PC2 memory test ($r=0.454$, $p=0.008$, $N=33$); while there were no significant correlations between variables of the exploration and memory tests. The absence of correlations of variables derived from different experiments suggests that these test measured personality traits that are relatively independent from one another, thus, leading to a conclusion of a lack of behavioural syndrome, for these measured personality traits in particular.



	Moderate stress	Exploration test			Memory test		
	PC	PC1	PC2	PC3	PC1	PC2	PC3
PC moderate stress	1.000	-0.204	0.197	0.401*	0.104	0.454**	-0.005
PC1 exploration test	-0.204	1.000	0.156	-0.154	0.301	-0.040	-0.031
PC2 exploration test	0.197	0.156	1.000	-0.129	-0.153	0.212	-0.251
PC3 exploration test	0.401*	-0.154	-0.129	1.000	0.239	0.030	0.310
PC1 memory test	0.104	0.301	-0.153	0.239	1.000	0.053	-0.056
PC2 memory test	0.454**	-0.040	0.212	0.030	0.053	1.000	0.083
PC3 memory test	-0.005	-0.031	-0.251	0.310	-0.056	0.083	1.000

Table 3: Pearson's correlations between principal components extracted for the moderate stress experiment and exploration and memory tests. Significant correlations are marked with an asterisk (* for $p < 0.05$; ** for $p < 0.01$).

A factor analysis on the original variables described within each personality experiment resulted in the extraction of two PCs explaining 53 % of the variance (Table 3). Thus, PC1 represented primarily variation in latencies in the exploration and memory test, indicating correlation between traits tested with those experiments, possibly due to the similarity of the experiments. Analyses using GLIM-model selection showed no significant effects for the factors (population, season, cohort nested within season, population-by-sex, population-by-season and season-by-sex interactions) (Supplementary table 3), confirming results obtained in the previous analyses using single variable (Supplementary table 2). High positive loadings of the variables of the moderate-stress experiment on PC2 and high to moderate negative loadings with of most of the variables obtained in the exploration test, suggest that this axis of behavioural variation represents a different, more complex component of the animal's personality. GLIM model selection on this principle component yielded similar results to those previously found analysing latencies of this experiments separately: namely, a significant effect of season (Wald $\chi_{(1)}=10.207$, $p=0.001$) and population (Wald $\chi_{(2)}=10.029$, $p=0.007$) (factors of population, season and population-by-sex interaction) (Table 4, Supplementary table 3). Moreover, PC2 was the variable that best separated populations (Figure 10).



Behavioural syndrome		PC1	PC2
% of variance		30.47	23.11
Eigenvalue		4.875	3.698
KMO measure of sampling adequacy		0.59	0.59
Bartlett's test of sphericity		<0.001	<0.001
Factor loadings			
Moderate stress experiment	<i>Latency to feed</i>	0.153	0.771
	<i>Latency to approach the usual feeder</i>	0.128	0.655
	<i>Total time spend in the usual feeder</i>	0.165	0.649
Exploration test	<i>Latency to feed from new feeder</i>	0.749	-0.489
	<i>Latency to approach the usual feeder</i>	0.669	0.055
	<i>Number of visits to the usual feeder</i>	0.297	-0.712
	<i>Total time spend in the usual feeder</i>	0.549	-0.554
	<i>Latency to approach the new feeder</i>	0.768	-0.440
	<i>Number of visits to the new feeder</i>	0.084	-0.556
Memory test	<i>Latency to feed from covered feeder</i>	0.853	0.235
	<i>Latency to approach the usual feeder</i>	0.389	0.314
	<i>Number of visits to the usual feeder</i>	0.820	0.105
	<i>Total time spend in the usual feeder</i>	0.765	0.337
	<i>Latency to approach the covered feeder</i>	0.816	0.252
	<i>Number of visits to the covered feeder</i>	0.090	0.096
	<i>Total time spent around the covered feeder</i>	0.067	0.585

Table 4: Factor loadings and percentage of explained variance, Keiser-Meyer-Olkin measure of sampling adequacy (KMO; >0.5) and Bartlett’s test of sphericity (<0.05) of the extracted “behavioural syndrome” principal components 1 and 2, from the variables extracted from the moderate stress experiment, and the exploration and memory tests. Factor loadings > 0.600 are marked in bold and loadings between 0.500 and 0.600 in italic.

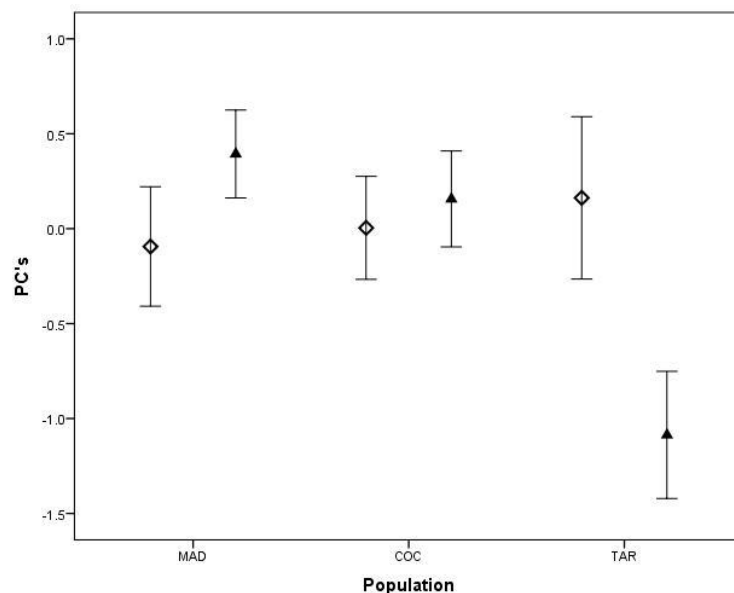


Figure 7: Mean (± 1 SE) factor scores extracted from all described personality variables; Behavioural syndrome PC1 marked by squares, PC2 marked by filled triangles; MAD – Madrid, COC - Cocentaina and TAR - Tarifa

10.3.6 Correlations between personality traits and migratory activity

Several significant correlations between personality traits variables and migratory activity were found. Firstly, the principal component of the “moderate stress” experiment is negatively correlated with all the variables for the onset of migratory activity (see Table 6). Secondly, exploration experiment results show tendency towards PC3 being negatively correlated with the onset of migratory activity. These results suggest that birds more affected by the moderate stress situation are the ones migrating earlier, as well as the ones more flexible in changing the food location (exploration test). In addition, regarding the amount of migratory activity, only tendency towards significant correlations were found, between the PC “moderate stress” and PC2 of the exploration test and the PC for the amount of higher migratory activity, implying that the birds with higher amounts of activity are as well less stressed within the moderate stress experiment and less exploratory of the unfamiliar feeder (see Table 7). No significant correlations were found between migratory behaviour and performance in the memory test.

	Moderate stress	Exploration test			Memory test		
	PC	PC1	PC2	PC3	PC1	PC2	PC3
PC onset	-0.527***	0.317	-0.027	-0.403 ⁺	0.081	-0.268	0.001
PC onset act1	-0.481***	0.121	-0.216	-0.318 ⁺	0.054	-0.276	0.089
PC onset act2	-0.388**	0.224	-0.191	-0.211	0.078	-0.077	0.21

Table 6: Pearson’s correlations between principal components extracted for the onset of migratory activity (PC onset, PC onset act1, PC onset act2-higher activity) and for the personality tests; moderate stress (PC); exploration (PC1, PC2, PC3) and memory test (PC1, PC2, PC3), $p < 0.001$ ***, $p < 0.01$ **, < 0.05 *, $p < 0.1$ ⁺

	Moderate stress	Exploration test			Memory test		
	PC	PC1	PC2	PC3	PC1	PC2	PC3
PC amount	0.191	-0.080	0.368	0.309	-0.045	0.102	-0.246
PC amount act2	-0.233 ⁺	0.165	-0.360 ⁺	-0.143	0.056	-0.140	0.133

Table 7: Table of Pearson’s correlations between principal components extracted for the amount of migratory activity (PC amount, PC amount act2-higher activity) and for the personality tests; moderate stress (PC); exploration (PC1, PC2, PC3) and memory test (PC1, PC2, PC3), $p < 0.001$ ***, $p < 0.01$ **, < 0.05 *, $p < 0.1$ ⁺

	Behavioural syndrome	
	PC1	PC2
PC onset	0.162	-0.580*
PC onset act1	0.045	-0.440*
PC onset act2	0.198	-0.401
PC amount	-0.097	0.018
PC amount act2	0.214	-0.169

Table 8: Table of Pearson’s correlations between principal components extracted for the onset and amount of migratory activity (PC onset, PC onset act1, PC amount, PC amount act2) and for the behavioural syndrome extracted principal components (Behavioural syndrome PC1 and PC2); $p < 0.001$ ***, $p < 0.01$ **, < 0.05 *, $p < 0.1$ ⁺



The extracted behavioural syndrome PC's were also tested for correlations with the described amount and onset of MA principal components, and only the PC2 of the behavioural syndrome proved to be correlated with the onset of the “lower” migratory activity (Table 8).

The analysis of covariance for the onset of migratory activity (PC onset) provided no evidence for a population-by-moderate-stress-experiment (PC) interaction effect ($F_{(2,48)}=0.047$, $p=0.954$). Also we found no evidence for interaction effects of population and other principal components summarizing personality traits on the onset of migratory activity (see Supplementary table 4). Moreover, no significant effects were found by the same interactions on the amount of migratory activity 2 PC variable (see Supplementary table 4).

Moreover, within population analysis showed that the slopes of within population regressions are similar to overall regression value for the correlation between PC moderate stress experiment latencies and PC onset of migratory activity (Madrid $R^2=0.166$, $B=-0.384$, $p=0.104$; Cocentaina $R^2=0.164$, $B=-0.367$, $p=0.040$; Tarifa $R^2=0.150$, $B=-0.496$, $p=0.238$; overall slope $R^2=0.278$, $B=-0.477$, $p<0.001$; weighted for the population sample size $N R^2=0.162$; Figure 8). Correlations between PC of the moderate stress experiment and PC of the higher amount of migratory activity show less similarity between within population slope and the overall slope value; Madrid $R^2=0.045$, $B=-0.172$, $p=0.413$; Cocentaina $R^2=0.125$, $B=-0.376$, $p=0.071$; Tarifa $R^2=0.007$, $B=0.138$, $p=0.804$; overall slope $R^2=0.054$, $B=-0.209$, $p=0.087$; weighted for the population $N R^2=0.077$ (Figure 9). Similar situation has been found for correlations between PC3 of the exploration experiment and PC of the onset of migratory activity (Madrid $R^2=0.921$, $B=-0.202.369$, $p=0.181$; Cocentaina $R^2=0.010$, $B=-0.108$, $p=0.701$; Tarifa $R^2=0.482$, $B=-0.534$, $p=0.126$; overall slope $R^2=0.268$, $B=-0.530$, $p=0.007$; weighted for the population $N R^2=0.231$; Figure 10) and PC2 of the exploration test and PC2 of the higher amount of migratory activity (Madrid $R^2=0.931$, $B=3.379$, $p=0.169$; Cocentaina $R^2=0.144$, $B=-0.600$, $p=0.133$; Tarifa $R^2=0.267$, $B=-0.553$, $p=0.239$; overall slope $R^2=0.129$, $B=-0.500$, $p=0.071$; weighted for the population $N R^2=0.263$; Figure 11).

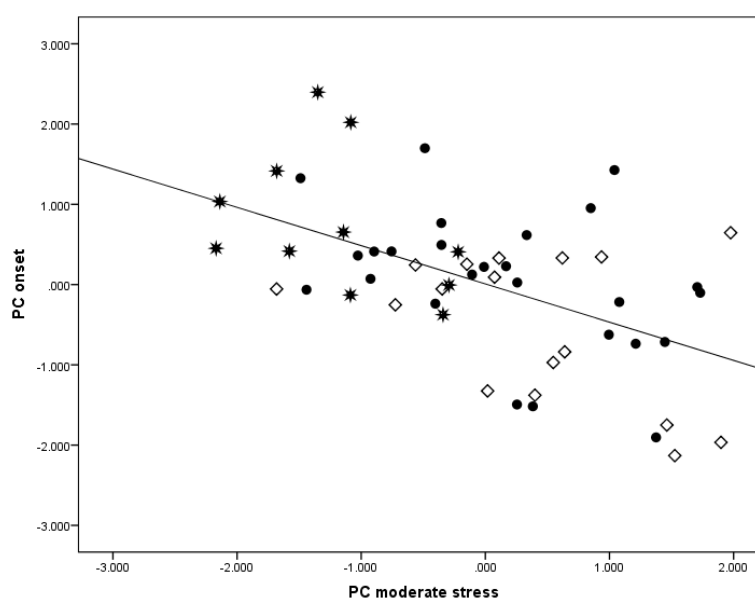


Figure 8: Scatter plot representing a regression line between PC of the onset of migratory activity and the PC of the moderate stress experiment, Madrid $R^2=0.166$, $p=0.104$, Cocentaina $R^2=0.164$, $p=0.040$, Tarifa $R^2=0.150$, $p=0.238$; overall slope $R^2=0.278$, $p<0.001$, weighted for the population $N R^2=0.162$; Madrid population is represented by squares, Cocentaina with filled in circles, Tarifa by filled in stars

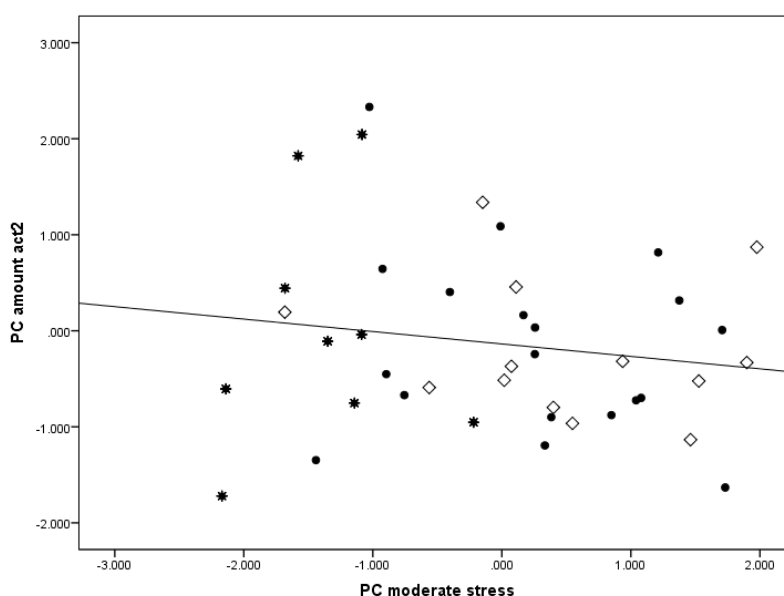


Figure 9: Scatter plot representing a regression line between PC of the higher amount of migratory activity and PC of the moderate stress experiment; Madrid $R^2=0.045$, $p=0.413$, Cocentaina $R^2=0.125$, $p=0.071$, Tarifa $R^2=0.007$, $p=0.804$; overall slope $R^2=0.054$, $p=0.087$; weighted for the population $N R^2=0.077$; Madrid population is represented by squares, Cocentaina with filled in circles, Tarifa by filled in stars

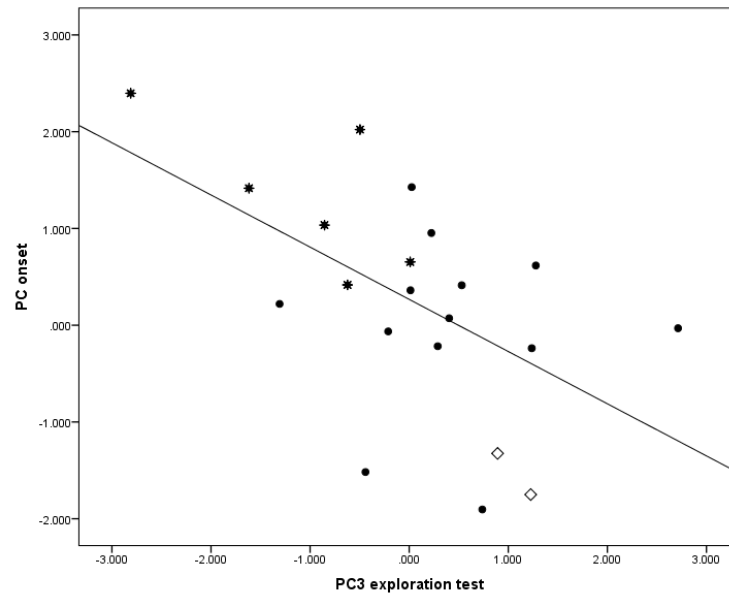


Figure 10: Scatter dot representing a regression line between PC of the onset of migratory activity and the PC3 of the exploration experiment; Madrid $R^2=0.921$, $p=0.181$, Cocentaina $R^2=0.007$, $p=0.701$, Tarifa $R^2=0.482$, $p=0.126$; overall slope $R^2=0.313$, $p=0.007$; weighted for the population N $R^2=0.231$; Madrid population is represented by squares, Cocentaina with filled in circles, Tarifa by filled in stars

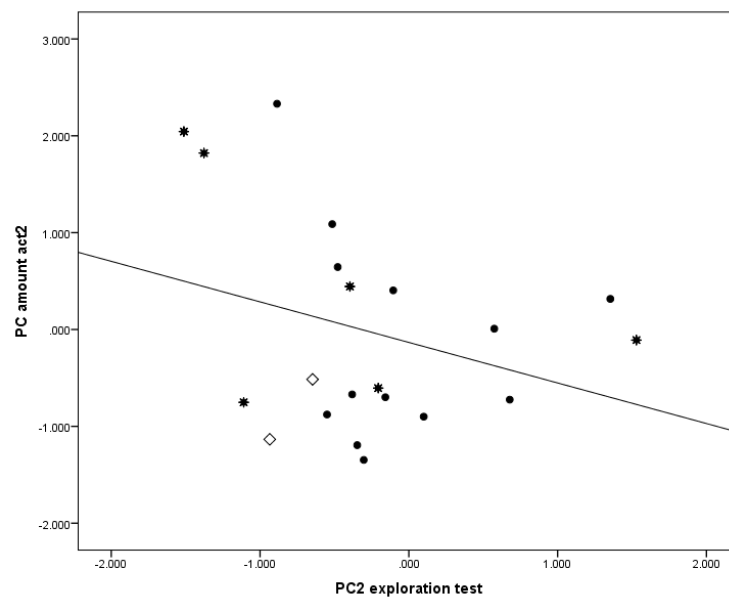


Figure 11: Scatter dot representing a regression line between PC of the higher amount of migratory activity and the PC2 of the exploration test; Madrid $R^2=0.931$, $p=0.169$, Cocentaina $R^2=0.144$, $p=0.133$, Tarifa $R^2=0.267$, $p=0.239$; overall slope $R^2=0.129$, $p=0.071$; weighted for the population N $R^2=0.263$; Madrid population is represented by squares, Cocentaina with filled in circles, Tarifa by filled in stars



10.4 Discussion

Behavioural syndromes are characterized by consistent individual behaviour across different situations, which results in significant correlations between different functional behaviours (e.g. bold birds being more aggressive, slow more neophobic etc.). In accordance with this prediction, we did find individual behaviour, however, repeatable (one of the characteristics of animal personalities). We would have also expected to find personality axes that would explain most of the variance in performance across tests. However, evidence for a behavioural syndrome for the traits measured here was not very strong, as we found only weak correlations among the variables measured in different experiments, and variation in these variables was not represented by one major axis. Principal component analysis revealed that about 50% of the variance was represented by two principle components: one PC reflecting variation of the variables measured in the exploration and memory tests and the other mainly representing variance in latencies of the moderate stress experiment, with some opposite contribution to the exploration test, suggesting that with our experiments we measured at least two different personality components. In addition, different behaviours can be measured with different errors and the contextual overlap among traits may also vary (Garamszegi *et al.*, 2013). Due to a substantial difference in correlated traits within the literature and variability in experimental designs, it is also possible that our experiments were not measuring traits representing the major personality axes in blackcaps, or we have chosen the wrong behaviours and contexts to study (Bell, 2007). As well, sometimes it is hard to distinguish between two behavioural traits within the same experiment, e. g. exploration and neophobia, as a novelty might evoke both exploration and neophobic reactions (Mettke-Hofmann *et al.*, 2002), and measuring different behaviours at the same time can be difficult (Garamszegi and Herczeg, 2012).

In this study we found significant population differences in personalities among three populations of Iberian blackcaps. The results suggest that sedentary population is coping better with moderate stress, compared to birds from the migratory population, which have longer latencies. This result may be explained by the sedentary way of life, permitting sedentary individuals to stay year-round in an environment with moderate changes. The strongest finding within the exploration test is that the sedentary Tarifa population is the least flexible population, the result being reflected in the shortest approaching times to the usual feeder and high rates of visits and time spent in it before feeding from the new plate (reflected as well within the behavioural syndrome PC2), characterising it as being more rigid in adapting to changes in food location than birds from the other populations. This may be due to the fact that in residents feeding behaviour need not be very innovative since they can rely on previous experiences. As they remain within the same area all-year round, food sources vary little in the course of the year and among years, and so it pays off to learn and remember them. In addition, in constant environments there is no need for flexible behavioural responses, as the stimuli do not change (Niemela *et al.*, 2013). On the other hand, birds from the Madrid and Cocentaina population proved to be more flexible in their behaviour, discarding faster the habit of visiting the familiar food source and directing their attention to the new one, confirming previous findings of an interspecific



study, in which migratory garden warblers were quicker in discovering food in novel environment compared to sedentary Sardinian warblers (Mettke-Hofmann and Gwinner, 2004). This characteristic could be beneficial for their life style – while migrating, foraging on stopover sites is time constricted, and hence a more flexible feeding behaviour and lower neophobia could be advantageous (Mettke-Hofmann *et al.*, 2009). These results, however, are in contradiction with results obtained in a study of 134 temperate Palaearctic Passerine species where resident species were more prone to evolve innovations, while migratory species were less flexible in their feeding behaviour (Sol *et al.*, 2005).

Our results showed higher flexibility in the migratory population. However, we cannot say much about the exploration, as difference were not statistically significant for the PC2. While the PC2 latencies are similar in value in the exploration test, it appears that Tarifa tends to have shorter exploring/neophobic latency in the memory test, possibly implying better memory of the food location in sedentary population (Figure 2, Chapter 4). However, as the literature states contradictory results regarding migratory styles and exploration rates, we should take into account differences in tested species and designs of experiments that could have contributed to the discrepancy in correlations of observed behavioural traits and migratory activity. Thus, our approach of testing two populations of the same species is valid; however, the possible limitations of experimental design may have masked personality differences.

As we have empirically measured migratory activity for each of our three populations, we have tried to correlate directly data from the personality experiments and the migratory restlessness results. Stress tolerance was negatively correlated with the onset of the migratory activity that differed significantly between populations (see Chapter 1), suggesting better stress tolerance within sedentary population. However, we should be careful with this interpretation, as the earlier onset does not necessarily characterize the more migratory population, as the earlier onset could be correlated to the earlier hatching date of the juveniles (Coppack *et al.*, 2001). The correlations between personality traits and the amount of migratory activity yielded only a tendency towards significant negative correlations. As there was no difference between populations in the amount of migratory activity (see Chapter 1), we cannot draw any conclusions regarding the distinction between personality types and their correlation with the amount of migratory activity displayed in captivity. The lack of a strong correlation between these two traits may be due to the fact that we have measured migratory activity in an artificial environment in which real differences in migratory behaviour may not be expressed. Another explanation could be the housing location of the birds and its environmental conditions, as we assume that the Madrid's migratory latitude could have induced the migratory behaviour within all three populations (Pulido, 2011) (see Chapters 1 and 2) causing the disappearance of personality types-migratory activity correlation.

It is also possible that there are no behavioural differences in exploration to detect within our study species or they are too subtle to be detected. The blackcap is a species in which all three migratory strategies (migratory, sedentary and partially migratory) are found, which suggest rapid evolutionary changes of those



strategies in response to the changing environmental conditions. We can state as an example a relatively newly established wintering areas within the Great Britain a few decades ago (Berthold *et al.*, 1992; Bearhop *et al.*, 2005), as well as the possibility of changing partially migratory populations to completely sedentary or migratory just in a few generations time (Pulido and Berthold, 2010). Due to this phenological flexibility of the species/populations, it is possible that prevailing personality types are the ones more flexible, capable of adjusting to different environmental conditions over relatively short period of time (few generations), excluding the extreme values of the personality axes within the populations. This hypothesis could be corroborated comparing repeatabilities with other studies. If, compared to other studies, repeatabilities are small and integration of traits is weak, it may indicate that animal personalities in blackcaps are less rigid.

The importance of this study is reflected within the intraspecific comparison of personality traits between populations of different migratory strategies, an approach scarcely represented within current literature. We believe that this is a more suitable approach for studying the relation between animal personalities and migration as it removes any possible confounding effects due to interspecific differences. Of course, possible limitations of the study do exist. For instance, there are no replicates for each of the populations representing one migratory strategy, thus, it can confound population differences non-related to migration and ones that are indeed present due to migratory behaviour. Difficulties could be produced as well by small among-population differences in migratory behaviour and the fact that those differences are most likely primarily environment-dependent.



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10.6 Supplementary tables

% of variance	Onset			Amount	
	PC	PC act1	PC act2	PC	PC act2
Eigenvalue	74.773	80.266	93.4	67.754	23.117
	3.739	2.408	1.869	5.42	1.849
Factor loadings					
Onset of MA 5 days criteria	0.828	0.816			
Onset of MA 5 days criteria higher activity	0.884		0.947		
Onset >3 intervals of activity in the central part of the night	0.940	0.948			
Onset >4 intervals of activity the central part of the night (act 2)	0.830		0.947		
Onset >3 intervals of activity, independent of continuity	0.836	0.918			
Average MA corrected for the maximum number of days of activity				0.866	-0.441
Sum of 30min activity corrected for days with more than >3 intervals and missing data				0.841	-0.48
Average MA corrected for the maximum number of days of higher activity				0.838	0.449
Sum of 30min activity corrected for days with more than >3 intervals and missing data (act 2)				0.758	0.569
Average MA corrected for the maximum number of days of activity (filled gaps)				0.87	-0.449
Sum of 30min activity corrected for days with more than >3 intervals and missing data (filled gaps)				0.836	-0.461
Sum of 30min activity corrected for days with more than >3 intervals and missing data (act 2, filled gaps)				0.747	0.567
Average MA corrected for the maximum number of days of higher activity (filled gaps)				0.818	0.404

Table 1: Factor loadings and percentage of explained variance for the principal component factor analysis of the onset of migratory activity and amount of first autumn migratory activity for the birds of the 2010, 2011 and 2012 cohorts



	Initial model		Final model (p<0.05)		Population	Season	Cohort(season)	Population*sex	Population*season	Sex*season
	AICC	BIC	AICC	BIC						
<i>PC moderate stress</i>	212.3	231.5	212.6	224.9	<0.001	<0.001	ni	ns	ni	ni
<i>Latency to feed (moderate stress)</i>	102.7	121.9	99.6	111.8	0.002	<0.001	ni	ns	ni	ni
<i>Latency to approach the feeder</i>	106.6	125.8	102.8	115.1	0.001	<0.001	ni	ns	ni	ni
<i>Total time spent in the feeder</i>	72.0	91.1	72.0	91.1	0.001	<0.001	ni	0.019	ni	ni
<i>Number of visits to the feeder</i>	213.6	232.8	214.3	221.8	ns	<0.001	ni	ns	ni	ni
<i>PC1 exploration test</i>	157.1	172.0	146.1	155.9	ns	0.061	0.002	ns	ni	ni
<i>PC2 exploration test</i>	148.4	162.9	140.7	152.7	0.045	ns	0.012	ns	ni	ni
<i>PC3 exploration test</i>	141.9	156.7	136.5	144.9	<0.001	0.023	ns	ns	ni	ni
<i>Latency to feed (exploration test)</i>										
<i>Latency to approach the new feeder</i>										
<i>Number of visits to the new feeder</i>	199.5	224.5	187.3	202.2	ns	ns	ns	ns	ns	ni
<i>Total time spent around new feeder</i>	98.3	120.2	88.4	101.4	ns	ns	ns	ns	ns	ni
<i>Latency to approach the usual feeder</i>	49.14	65.4	39.1	51.6	0.003	0.01	0.004	ns	ns	ni
<i>Total time spent in the usual feeder</i>	103.3	118.7	88.15	101.1	0.066	ns	ns	ns	0.053	0.022
<i>Number of visits to the usual feeder</i>	59.0	81.1	53.1	66.2	ns	ns	0.001	ns	ni	ni
<i>PC1 memory test</i>	164.7	178.7	154.9	167.3	ns	ns	ns	ns	ns	ns
<i>PC2 memory test</i>	153.7	167.9	132.3	140.4	ns	<0.001	0.067	ns	ns	ns
<i>PC3 memory test</i>	166.4	180.5	150.6	163.0	ns	ns	ns	ns	ni	ns
<i>Number of visits to the covered feeder</i>	150.0	175.1	137.0	147.6	ns	0.028	ns	ns	ns	0.033
<i>Total time spent around covered feeder</i>	67.2	92.0	56.5	70.8	ns	ns	0.001	ns	ns	0.031
<i>Latency to approach the usual feeder</i>	90.6	105.4	68.7	76.9	ns	ns	ns	ns	ni	ns
<i>Total time spent in the usual feeder</i>	124.1	138.9	102.1	110.3	ns	ns	ns	ns	ni	ns
<i>Number of visits to the usual feeder</i>	342.0	356.2	323.8	334.3	ns	0.012	0.001	ns	ni	0.008

Table 2: Corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC) as selection criteria for the final GLIM model for the analysis of different factors' effects on the original variables and principal components extracted for each behavioural test. The initial model includes all the variables stated within the table, except for the "ni" (not included) within some cases, and the final model contains the variables with the significant p value (or a tendency towards it). Significant effects are stated by its p value, ns stands for a non-significant effect, ni is a factor not included into analysis due to a previously conducted exploratory model analysis.

	Initial model AICC, BIC	Final model AICC, BIC	Population	Season	Cohort(season)	Population*sex	Population*season	Sex*season
PC1 behavioural syndrome	124.4; 121.6	97.9; 103.1	ns	ns	ns	ns	ns	ns
PC2 behavioural syndrome	85.4; 90.4	79.3; 84.1	0.007	0.001	ni	ns	ni	ni

Table 3: Corrected Akaike information criterion (AICC) and Bayesian information criterion (BIC) as selection criteria for the final GLIM model for the analysis of different factors' effects on the original variables and principal components extracted representing behavioural syndrome. The initial model includes all the variables stated within the table, except for the "ni" (not included) within some cases, and the final model contains the variables with the significant p value (or a tendency towards it). Significant effects are stated by its p value, ns stands for a non-significant effect, ni is a factor not included into analysis due to a previously conducted exploratory model analysis.

	PC onset	PC amount 2
Population * PC moderate stress	$F_{(2,48)}=0.047$, $p=0.954$	$F_{(2,49)}=0.640$, $p=0.532$
PC1 exploration test	$F_{(2,20)}=0.895$, $p=0.424$	$F_{(2,20)}=1.498$, $p=0.248$
PC2 exploration test	$F_{(2,20)}=2.515$, $p=0.106$	$F_{(2,20)}=0.839$, $p=0.447$
PC3 exploration test	$F_{(2,20)}=0.967$, $p=0.397$	$F_{(2,20)}=0.628$, $p=0.544$
PC1 memory test	$F_{(2,19)}=0.406$, $p=0.672$	$F_{(2,19)}=1.905$, $p=0.176$
PC2 memory test	$F_{(2,19)}=2.018$, $p=0.160$	$F_{(2,19)}=0.956$, $p=0.402$
PC3 memory test	$F_{(2,19)}=0.051$, $p=0.951$	$F_{(2,19)}=0.529$, $p=0.598$

Table 4: Population-by-principal-component (extracted for each of the personality experiments) interaction effects on the principal components representing the onset and the amount (higher) of migratory activity.





11 Chapter 4

**“Long-term memory in blackcaps (*Sylvia atricapilla*)
differing in migratory behaviour”**

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11.1 Introduction

The ability of an individual to acquire, process and use information has evolved in response to the large amount of information coming from alternative environments (Niemela *et al.*, 2012), memorising it over long periods of time (Cook *et al.*, 2005; Weiss and Scheiber, 2013). This holds particularly true for migratory birds, which every year conduct a return journey between their breeding and wintering sites and which during this trip are challenged with threats of starvation, predation or of getting lost. It is believed that those changing conditions are driving the evolution of cognition, where more unstable environments favour higher cognitive capability (Bergman and Feldman, 1995), resulting in differences in the efficient use of environmental information between individuals, populations and species (Wolf *et al.*, 2008). A good example for this are the numerous species of migratory birds, which manage to return to the same wintering and breeding grounds year after year in a regular seasonal migration (Moreau, 1972; Winkler *et al.*, 2014).

Inexperienced passerines use the sun, stars or the Earth's magnetic field (Alerstam, 2006) as compass, where the routes used and distances covered during the first migratory journey are probably innate (Wiltschko and Wiltschko, 1988, Berthold, 1996, Pulido, 2007) in species of small passerines which do not migrate in flocks (Berthold, 2001). Compared to resident species, migrants tend to have better spatial and long-lasting memory, which helps them to optimize their journey, playing an important role in navigation and goal finding (Mettke-Hofmann and Gwinner, 2003). While migrating, suitable stopover sites and landmarks are memorized along the way (Wiltschko and Wiltschko, 1988; Burt *et al.*, 1997; Kamil and Cheng, 2001) facilitating orientation and finding the previously used breeding or wintering areas or stopover sites (Mettke-Hofmann and Gwinner, 2003). This presumption is supported by experiments in which experienced migrants were translocated from their migratory routes and released at a site they did not know. These experiments showed that migratory birds were able to return to the sites of capture (Bingman *et al.*, 1990; Berthold, 1994). Those kind of differences in cognitive ability may affect differences in behavioural flexibility (Sol *et al.*, 2005), in the way that more efficient processing of the information gathered in new and uncertain situations results in a more efficient behavioural response to that information.

Here, we tested for differences in cognitive abilities among two Iberian populations of blackcaps (*Sylvia atricapilla*), that in the wild differ in migratory tendency. Specifically, we studied long-term memory effects, based on personality experiments which, in addition to other behavioural traits, tested for differences in cognition. We predicted that individuals from the migratory populations would have a longer memory than sedentary individuals.



11.2 Materials and methods

11.2.1 Studied animals

This study is a part of an extensive personality study (see Chapter 3) on three Iberian Blackcap populations. We selected these populations because they differ in migratory behaviour within a small geographic area. Specifically, the sampling locations used in this study were Madrid, as the location of migratory population (Tellería *et al.*, 2001) (Pinilla del Valle 40°55'N, 3°49'W) and Cocentaina as partially migratory population (Morganti *et al.*, 2015) (38°44'N, 0°26'W). Juveniles 2-3 months old, of both sexes, were captured in summer of 2011 (N=33), between 15th of July and 2nd of August. In this study we considered only birds from Madrid and Cocentaina that were tested both in autumn 2011 and in spring 2012 (N=24; 12 from Madrid, 7 males and 5 females; 12 from Cocentaina, 9 males and 3 females). Upon capture, each bird was assigned its own code in colour rings so it could be easily identified. After capture, birds were transported to our study facility in the restricted area within the Madrid's natural park (Casa de Campo, 40°25'N, 3°45'W). The birds were kept inside individual cages (45x23x38cm) with food and water *ad libitum* (for more detailed description of housing conditions, see Pulido and Coppack, 2004). They had visual contact with individuals in neighbouring cages, and auditory contact with all individuals. After the experimental period was finished, at the end of May of each experimental year, all the individuals were released after removal of colour rings at the exact sites where they had been captured originally. Three “personality defining” experiments were conducted within autumn and spring migratory seasons. The long-term memory was considered in the context of ecologically relevant time periods, lasting from weeks to months (Roth *et al.*, 2012).

11.2.2 Tests for testing cognitive capacity

The first experiment was similar to the usual maintenance protocol. Yet, in this experiment, birds were deprived of food by removing the feeder for 20-30min, creating a mildly stressful situation. After returning the feeder, birds would approach the feeder and feed after some latency time, which depends on the response to the experiment.

In the second experiment (exploration test), after same food deprivation period a new unfamiliar feeder (clay plate, Ø 7cm, 2cm high) was introduced into the cage, while the familiar feeder remained empty. Latency to approach and feed was measured as well.

The third experiment (memory test) was identical to the second experiment, but here the newly introduced feeder was covered with a piece of paper, hiding the food. This kind of setup demanded from the birds the cognitive ability to remember and recognize the feeder and to search for food under the paper. In this experiment the same parameters were measured as in the second experiment. For details, see description of the experiments in Chapter 3.



To study the effect of learning, each of the three experiments were repeated six times: three times during the bird's first autumn migration and three times during its first spring migration. Within each season, experiments were repeated every 2-3 days. Time between migratory seasons (i.e. between the 3rd and 4th repetition) was 116-135 days. The experimental setup was exactly the same in every test. We considered only individuals which were tested in both migratory seasons (cohort 2011). Individuals not completing an experiment were excluded from the corresponding analyses (Supplementary Table 1). The variables studied in each test and described within the following results were predominantly the latencies to feed associated with stress, neophobia and learning, chosen because we expected to observe the learning effect within them. Latency to approach the usual feeder and time spent in it was not considered in the analysis as the majority of individuals who approached it did not finish the experiment, and were thus excluded. In contrast, only few individuals that accomplished the assignment visited the usual feeder. To have a measure of the learning effect we calculated linear regression coefficient for the individual's latency to feed throughout two seasons (6 repeats) and for each season separately (3 repeats; within season coefficients). Additional linear regression coefficient was calculated for the same latency variables, but based on the number of the days elapsed since the beginning of the experiment (1-146 days). The results based on the two linear regression coefficients did not differ. Here, we present only results using the linear regression by order of repeat. In all analyses, the effect of "sex" was non-significant. We, therefore, did not further consider it in the models. Statistical analyses were conducted using SPSS, version 20 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

11.3 Results

11.3.1 Population effects

GLIM model analysis displays significant effect of repeats for all three experiments, demonstrating a decrease in mean latency to feed throughout the three personality experiments (Table 1; Graph1).

Regarding the differences within seasons, only within the moderate stress experiment, populations differ in the mean linear regression coefficient (B) (Wald $\chi^2_{(1)}=6.451$, $p=0.011$)(Table 1), where Madrid and Cocentaina differ in the mean latency in the 3rd repeat ($F_{(1,21)}=6.461$, $p=0.019$; mean Madrid latency 1.44, mean Cocentaina latency 1.87).



OVERALL	Starting model	Final model (p<0.05)	Factors		
	AICC; BIC	AICC, BIC	Population	Repeat	Population*Repeat
Latency to feed from the usual feeder (moderate stress test)	131.4; 145.7	128.1; 136.7	ns	<0.001	ns
Latency to feed from the new feeder (exploration test)	88.9; 102.3	85.1; 93.2	ns	<0.001	ns
Latency to approach the covered feeder (memory test)	122.7; 132.7	119.8; 127.7	ns	0.001	ns
AUTUMN					
Latency to feed from the usual feeder (moderate stress test)	81.1; 91.1	81.5; 89.8	ns	<0.001	0.011
Latency to feed from the new feeder (exploration test)	47.7; 56.8	43.1; 48.8	ns	<0.001	ns
Latency to approach the covered feeder (memory test)	65.1; 73.5	62.0; 67.3	ns	0.029	ns
SPRING					
Latency to feed from the usual feeder (moderate stress test)	45.9; 56.3	42.6; 49.0	ns	0.014	ns
Latency to feed from the new feeder (exploration test)	47.1; 56.8	44.8; 50.8	ns	<0.001	ns
Latency to approach the covered feeder (memory test)	66.7; 75.9	66.7; 75.9	ns	ns	ns

Table 1: Table of AICC (corrected Akaike information criterion) and BIC (Bayesian information criterion) values for the final GLIM model over all 6 repeats and within each season with population, repeat and population-by-repeat interaction as factors included in the model. For each significant effect p values are given. ns indicates that an effect is non-significant.

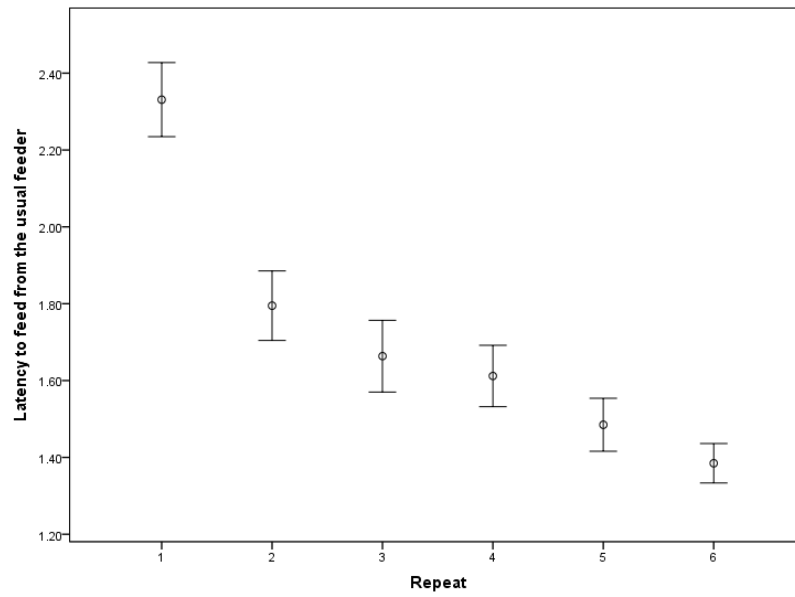
To test whether long-term memory is found in all three personality experiments, the mean latency values of the 3rd and the 4th replica were compared for possible difference in means for each population separately, where the absence of the significant effect would be an indicator for a memory effect (Table 3, a)). In addition, mean latency values were compared between populations within both 3rd and 4th replica, and it was found only for the moderate stress experiment's feeding latency to have different population means, where Madrid had lower mean latency within the 3rd repeat ($F_{(1,21)}=6.641$, $p=0.019$) (Table 2, b)).

a)	Within population difference from 3rd to 4th repeat	Madrid	Cocentaina
	Latency to feed from the usual feeder (moderate stress test)	$F_{(1,21)}=1.084$, $p=0.310$	$F_{(1,22)}=3.023$, $p=0.096$
	Latency to feed from the new feeder (exploration test)	$F_{(1,16)}=0.768$, $p=0.394$	$F_{(1,22)}=0.227$, $p=0.638$
	Latency to approach the covered feeder (memory test)	$F_{(1,14)}=0.010$, $p=0.922$	$F_{(1,22)}=0.1057$, $p=0.749$
b)	Within 3rd and 4th repeat difference between populations	3rd repeat	4th repeat
	Latency to feed from the usual feeder (moderate stress test)	$F_{(1,21)}=6.641$, $p=0.019$	Mad mean 1.44; Coc mean 1.87 $F_{(1,22)}=0.027$, $p=0.872$
	Latency to feed from the new feeder (exploration test)	$F_{(1,19)}=0.109$, $p=0.744$	$F_{(1,19)}=0.965$, $p=0.338$
	Latency to approach the covered feeder (memory test)	$F_{(1,17)}=0.604$, $p=0.448$	$F_{(1,19)}=2.580$, $p=0.125$

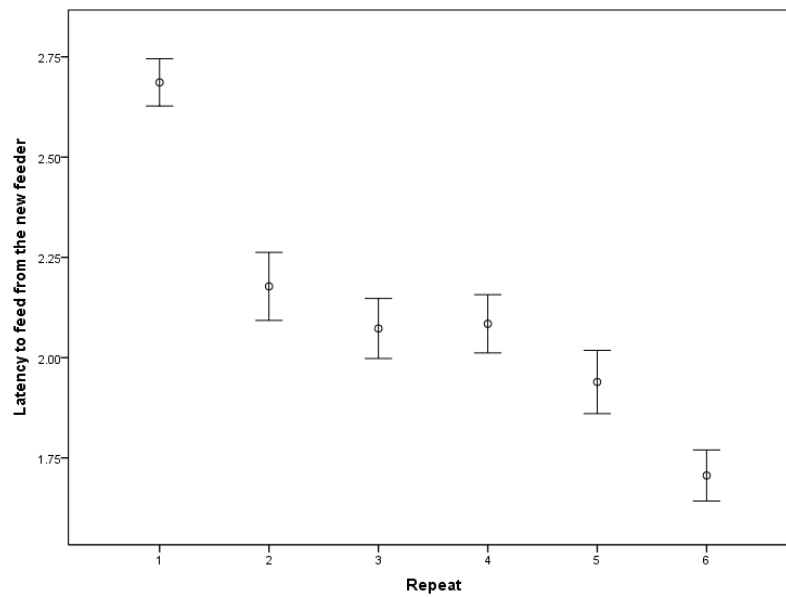
Table 2: ANOVA's statistics for the latency to feed of each of the three personality experiments demonstrating a) differences within Madrid and within Cocentaina populations of the mean latency values from the 3rd to the 4th repeat; b) differences between Madrid and Cocentaina population's mean latency within the 3rd and within the 4th repeat; Mad – Madrid, Coc – Cocentaina

Additionally, there is also no significant difference between latencies in the 3rd and 4th repeat of the moderate stress experiment within populations calculated by paired t-test (Madrid $t_{(10)}=-1.396$, $p=0.193$ (two-tailed); Cocentaina $t_{(11)}=1.944$, $p=0.078$ (two-tailed)); none in the exploration test (Madrid $t_{(7)}=-0.941$, $p=0.378$ (two-tailed); Cocentaina $t_{(11)}=0.544$, $p=0.597$ (two-tailed)) and none for the memory test (Madrid $t_{(5)}=-1.156$, $p=0.300$ (two-tailed); Cocentaina $t_{(8)}=0.639$, $p=0.541$ (two-tailed)).

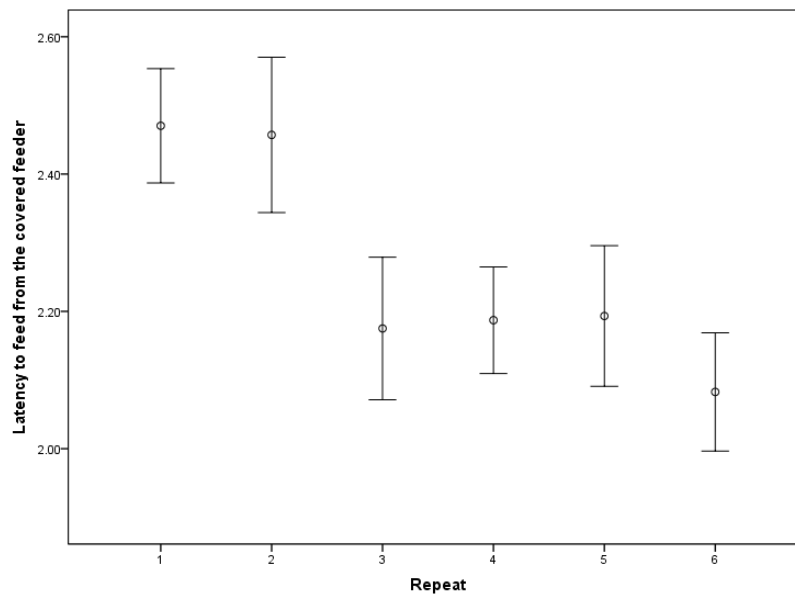
Individual-by-repeat interaction was significant for the latency to feed in the “moderate stress experiment” (Wald $\chi_{(23)}=45.482$, $p=0.003$) and in the exploration test (Wald $\chi_{(22)}=142.9$, $p<0.001$), but not in the memory test (Wald $\chi_{(20)}=13.708$, $p=0.845$). This demonstrates among-individual differences in the change of latency in two of the three experiments (Supplementary Table 2, Supplementary Graphs 1, 2 and 3).



Graph 1: Mean ± 1 latency to feed (log transformed seconds) from the usual feeder through 6 repeats within the moderate stress experiment for the pooled Madrid and Cocentaina populations (1-3 autumn; 4-6 spring).



Graph 2: Mean ± 1 latency to feed (log transformed seconds) from the new feeder through 6 repeats within the exploration test for the pooled Madrid and Cocentaina populations (1-3 autumn; 4-6 spring).



Graph 3: Mean ± 1 latency to feed (log transformed seconds) from the covered feeder through 6 repeats within the memory test for the pooled Madrid and Cocentaina populations (1-3 autumn; 4-6 spring).

11.3.2 Learning effect with time or repeat

Linear regression coefficient for latency to feed throughout all 6 repeats of the moderate stress (B moderate stress) did not show any difference between Madrid and Cocentaina (ANOVA $F_{(1,20)}=0.302$, $p=0.589$). Additionally, the regression coefficient for the moderate stress experiment calculated separately within seasons differs significantly between autumn and spring (ANOVA $F_{(1,45)}=12.075$, $p=0.001$) with a stronger reduction of latencies in autumn than in spring (autumn mean B -0.35; spring mean B -0.11). Looking separately into seasons, only within autumn birds of the two populations differed in the mean linear regression coefficient (ANOVA $F_{(1,21)}=6.248$, $p=0.021$), where Madrid has significantly lower values (autumn Madrid mean B -0.48; spring mean B -0.23) while they coincide in values within spring.

Linear regression coefficient for the variable “latency to feed from the new plate” (B exploration test) fitted to the 6 repeats of the exploration test did not statistically differ among populations ($F_{(1,20)}=0.268$, $p=0.611$). Regression coefficients for the exploration test calculated within seasons differed significantly between autumn and spring as well (ANOVA $F_{(1,42)}=10.820$, $p=0.002$) with more negative values within autumn (autumn mean B -0.33; spring mean B -0.11). This among-season difference in the reduction of latencies was also found within each of the two populations studied (Madrid $F_{(1,18)}=5.037$, $p=0.038$; Cocentaina $F_{(1,22)}=5.337$, $p=0.031$) values.

Linear regression coefficient for the variable “latency to feed from the covered plate” (B memory test) throughout all 6 repeats of the memory test showed no difference between populations ($F_{(1,17)}=2.029$, $p=0.172$). B memory test calculated within seasons has more negative mean value within autumn ($F_{(1,40)}=4.447$, $p=0.041$; autumn mean B -0.173; spring mean B -0.02), however, the two populations did not



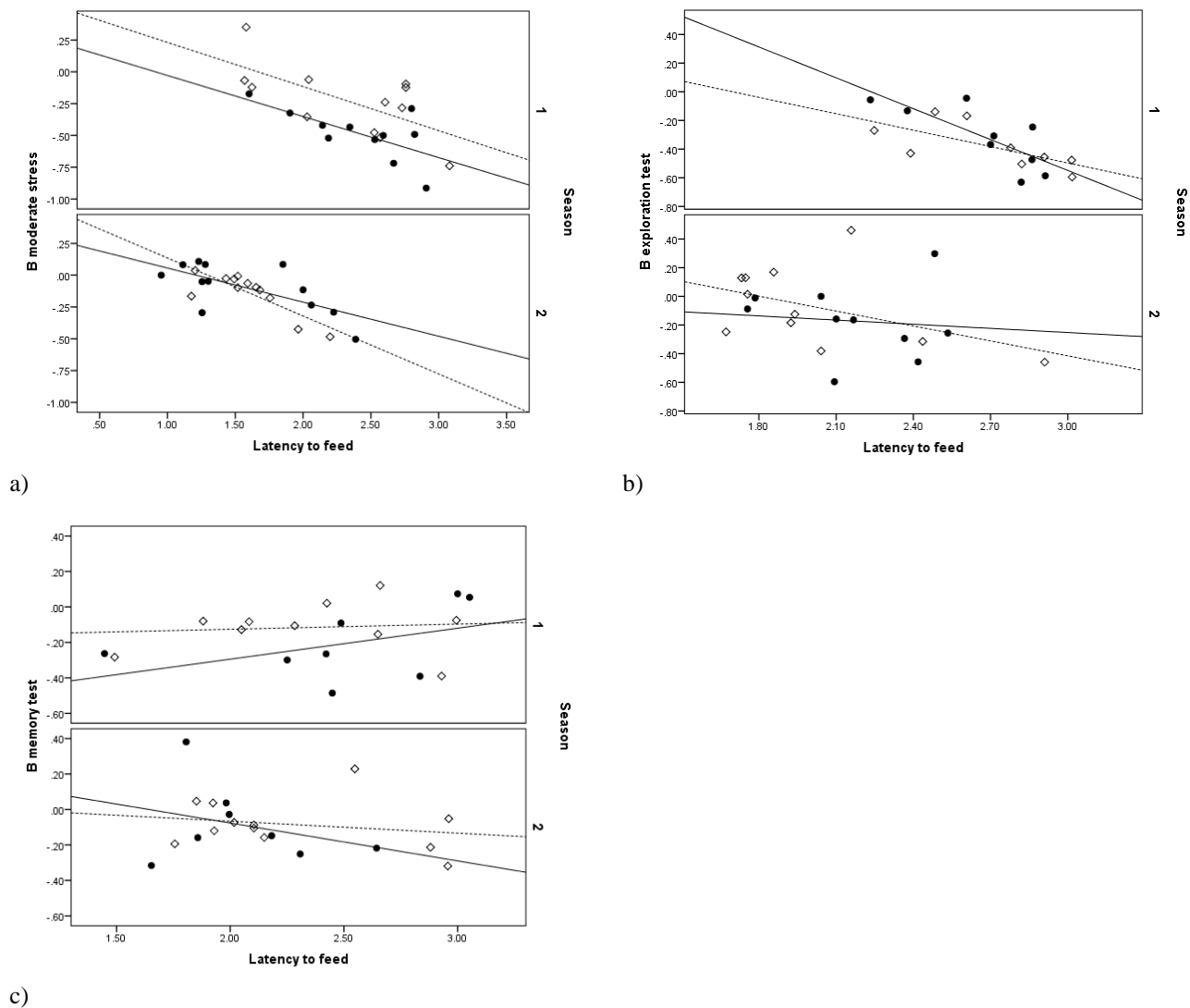
show any differences within each of the seasons (autumn $F_{(1,18)}=0.798$, $p=0.384$; spring $F_{(1,20)}=1.482$, $p=0.238$).

11.3.3 Relation of learning

B moderate stress plotted against latency to feed from the usual feeder in the first repeat, showed a negative correlation in autumn ($B=-0.339$, $p=0.005$, $N=22$), and in spring as well ($B=-0.317$, $p<0.001$, $N=24$), indicating that the birds with longer initial latencies (i.e. more affected by stress) more strongly reduced latencies in subsequent repeats.

The second B exploration test plotted against latency to feed from the new feeder within the first repeat, showed a negative correlation in autumn as well ($B=-0.526$, $p=0.001$, $N=18$), while there was none found for spring ($B=-0.286$, $p=0.112$, $N=21$).

No significant correlations were found between latency to feed from the covered plate within the first repeat and the linear regression coefficients B memory test.



Graph 2: Rate of reduction of the latency to feed as a function of the initial latency to feed in (a) the moderate stress experiment (Madrid: autumn (season 1) $R^2=0.436$, spring (season 2) $R^2=0.460$; Cocentaina: autumn $R^2=0.433$, spring $R^2=0.665$), (b) the exploration test (Madrid: autumn $R^2=0.596$, spring $R^2=0.011$; Cocentaina: autumn $R^2=0.464$, spring $R^2=0.211$) and (c) the memory test (Madrid: autumn $R^2=0.196$, spring $R^2=0.093$; Cocentaina: autumn $R^2=0.010$, spring $R^2=0.045$). Madrid is marked with filled dots and straight line, Cocentaina marked with empty squares and broken line, Season 1-autumn, season 2-spring, Latency to feed-latency to feed in each of the three experiments, B moderate stress experiment, B exploration test and B memory test-linear regression coefficients for the latency to feed through replicas within each season.

11.4 Discussion

During two consecutive seasons (autumn and spring) we conducted a series of controlled behavioural experiments and found evidence for learning and long-term memory of the learned behaviour among seasons. Within all three experiments conducted, the latency to feed significantly decreased from the first experiment in autumn to the last one in spring. We also found that in all experiments latencies did not significantly change between the last experiment in autumn and the first experiment in spring, strongly suggesting that birds did remember the information acquired 4 months earlier.



The decrease in latency after a moderately stressful situation could be interpreted as a result of habituation, not learning, to the conditions in cage. Nevertheless, habituation itself is a form of learning (Rankin *et al.*, 2009). For example, an animal that has more efficient information processing would habituate sooner to the new object/situation and explore it more extensively (Light *et al.*, 2011). We found that the completely migratory Madrid population showed a stronger reduction of latencies within autumn compared to Cocentaina, which could indicate better stress tolerance. If we consider this result in the light of migratory life style, it seems adaptive for migrants to be less affected by stress in every day performances as they are exposed to numerous stressful situations along their migratory route (Wingfield, 1994; Marra and Holberton, 1998; Landys-Ciannelli *et al.*, 2002).

The continuous decrease in latency confirms persisting memory of the tasks from one migration season to another, indicating that learned behaviour is maintained at least until spring. Looking at the linear regression coefficients, it appears that the learning process for both populations is stronger within autumn, or during the first few repeats.

Evidence for the long term memory in birds was found in previous studies, the evidence coming from food-hoarding species (Balda and Kamil, 1992; Clayton and Dickinson, 1999), hummingbirds (Henderson *et al.*, 2001) and pigeons (Cook *et al.*, 2005). Roth and colleagues (Roth *et al.*, 2012) found that small birds like parids, in this case the black-capped chickadee (*Poecile atricapillus*), can remember the location of a single food item for at least 6 months, maintaining spatial memories for long time, previously only found in corvids (Balda and Kamil, 1992). Probably the clearest example of high memorizing capacity in a migratory bird is found in a study on the garden warbler (*Sylvia borin*), a long distance migrant, which demonstrated the ability to memorize and remember a particular feeding site for at least a year. In contrast, the Sardinian warbler (*Sylvia melanocephala momus*), which is non-migratory species in the same genus, wasn't able to retain this information for more than two weeks (Mettke-Hofmann and Gwinner, 2003). The researchers claimed that their findings suggest long-term memory as an important factor in successful migration, by helping birds to remember the location of quality stopover sites and migration routes. This, they suggested, should be particularly important in older birds that use a more complex orientation system based on memory and learning (Mettke-Hofmann and Gwinner, 2003). This conclusion could apply to our migratory population as well. In our study, however, we demonstrated presence of long-term memory in short distance migrants, a result that is completely new and yet in accordance to the previously stated results for the long distance migrants.

Our study was not testing spatial memory per se, but the learning of adequate behaviour (i.e. reduction of stress response under a non-threatening situation, loss of neophobia and food placement). We were able to demonstrate the longevity of this learned adaptive behaviour within the context of only six repeated trials over the period of four months. For the difference between migratory and partially migratory populations, we found that migrants cope better with stress over the course of time and adjust to the situation more rapidly, at least



during the autumn migratory season, but we did not find any evidence for difference in long-term memory. In order to confirm these results and to broaden the knowledge of the long term memory effect of populations with different migratory strategies, we should include in the future analysis higher sample size for the completely sedentary population in order to compare the possible learning rate and memory retention differences. As well, we recommend studying memory in birds in other (different) contexts than described here and testing whether there are memory differences in birds from different populations differing in migratory propensity on a longer time scale (e.g. 1 year).



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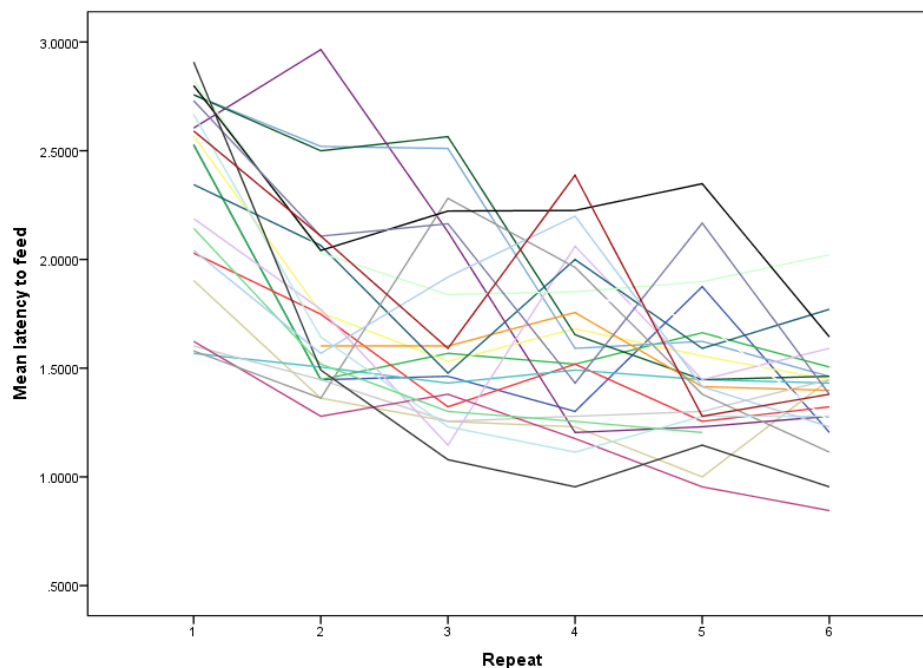
11.6 Supplementary material

		Moderate stress		Exploration test		Memory test	
		Mad	Coc	Mad	Coc	Mad	Coc
Males	autumn	6	8	5	5	4	7
	spring	7	9	7	9	6	9
Females	autumn	5	3	4	4	3	3
	spring	5	3	2	3	3	3

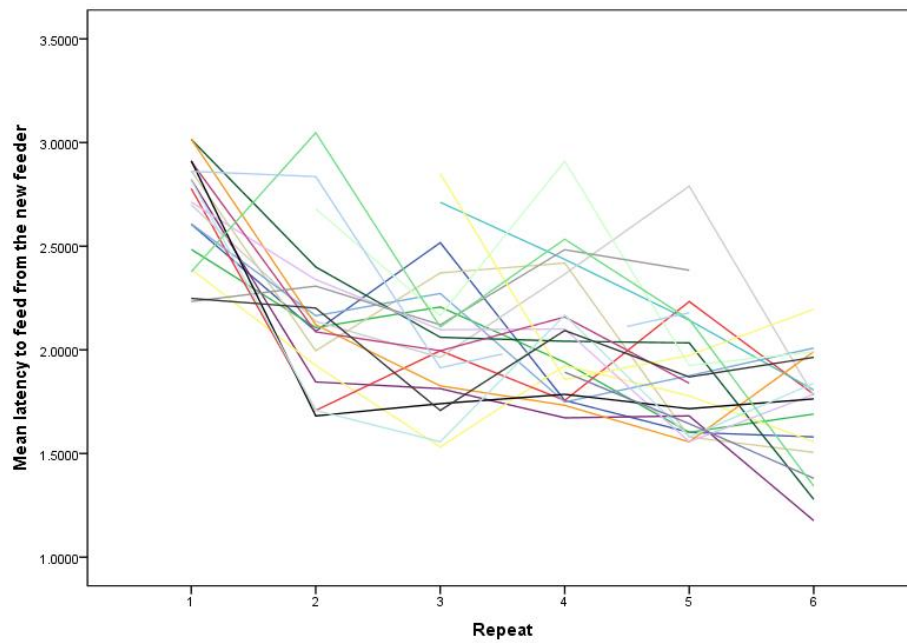
Table 1: Number of individuals within each population/season after excluding ones that have failed to fulfil each experiment's assignment within the given time of 20:00min.

	Starting model	Final model ($p<0.05$)	Individual(population)	Factors	
	AICC, BIC	AICC, BIC		Repeat	Individual*Repeat
Latency to feed from the usual feeder (moderate stress test)	177.2; 265.9	177.2; 265.9	$p<0.001$	$p<0.001$	$p=0.003$
Latency to feed from the new feeder (exploration test)	154.5; 224.6	90.9; 147.1	ns	$p<0.001$	$p<0.001$
Latency to approach the covered feeder (memory test)	166.2; 224.4	90.7; 143.1	$p<0.001$	$p<0.001$	ns

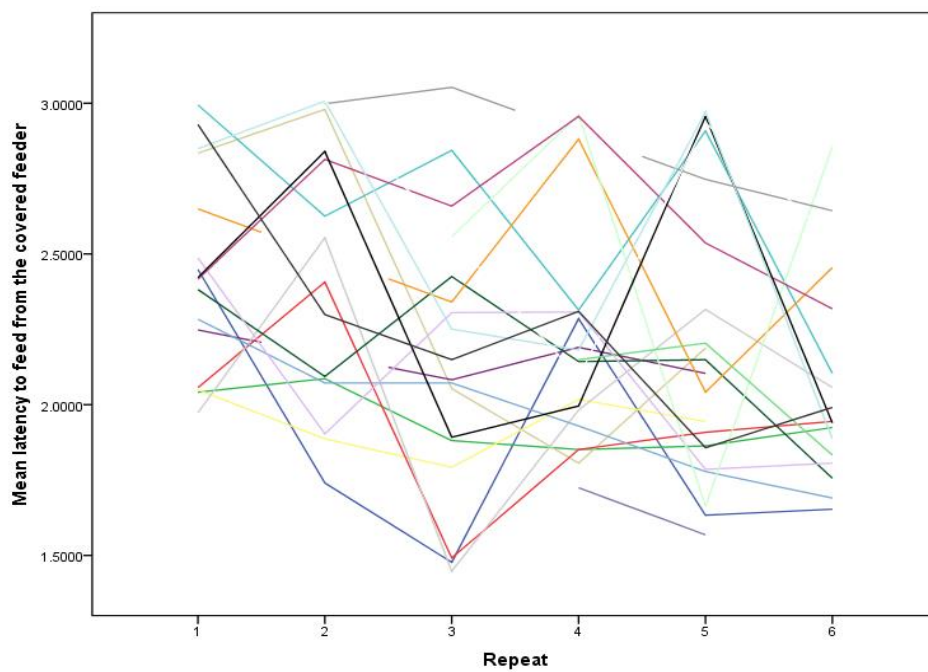
Table 2: Table of AICC (corrected Akaike information criterion) and BIC (Bayesian information criterion) values as the analysis selection criteria for the final GLIM model for latency to feed of the three personality experiments, with individual(population), repeat and individual-by-repeat interaction as factors. Significant effects are stated by its p value, ns stands for a non-significant effect



Graph 1: Mean latency to feed after a mildly stressful situation (moderate stress experiment) showed for each individual over the whole experimental period, from the 1st to the 6th repeat, N=24



Graph 2: Mean latency to feed from the newly introduced feeder (exploration test) showed for each individual over the whole experimental period, from the 1st to the 6th repeat, N=33



Graph 3: Mean latency to feed from the new covered feeder (memory test) showed for each individual over the whole experimental period, from the 1st to the 6th repeat, N=23



Individual	Population	Season	Moderate stress		Exploration test		Memory test	
			B between seasons	B overall	B between seasons	B overall	B between seasons	B overall
c34	1	1	-0.533	-0.157	-0.045	-0.2100	-0.486	-0.1
c36	2	1	-0.478	-0.129	-0.139	-0.1640	-0.08	-0.037
c37	1	1	-0.324	-0.097	-0.246	-0.2280	-0.391	-0.247
c38	2	1	-0.239	-0.364	-0.504	-0.2530	-0.083	-0.029
c39	2	1	-0.518	-0.173	-0.429	-0.1200	-0.128	-0.008
c40	2	1	-0.354	-0.138	-0.391	-0.1040	-0.283	-0.048
c41	2	1	-0.068	-0.023	-0.184	-0.3010	-0.075	-0.118
c42	1	1	-0.173	-0.034	-0.368	-0.0640	-0.263	0.007
c45	2	1	-0.123	-0.288	-0.168	-0.1250	-0.106	-0.114
c47	2	1	-0.096	-0.301	-0.477	-0.2800	0.021	-0.093
c48	2	1	-0.739	-0.060	-0.595	-0.1980	-0.154	-0.058
c49	1	1	-0.521	-0.087	-0.308	-0.1990	-0.091	-0.107
c50	1	1	-0.434	-0.108				
c51	1	1	-0.492	-0.125				
c53	2	1	-0.121	-0.145	-0.458	-0.1620	-0.261	-0.04
c57	2	1	0.351	-0.074	-0.456	-0.2070	0.121	-0.029
c58	1	1	-0.289	-0.139	-0.056	0.0480	0.054	-0.101
c59	1	1	-0.719	-0.233	-0.586	-0.1600	-0.265	-0.056
					-0.631	-0.1340	-0.299	-0.143
c61	2	1	-0.283	-0.209	-0.115	-0.1850	-0.262	-0.054
c63	1	1	-0.421	-0.214		-0.2560		
c64	1	1	-0.914	-0.312	-0.133	-0.2130		
c65	2	1	-0.061	-0.121	-0.27	-0.0580	-0.39	-0.167
c66	1	1	-0.5	-0.221	-0.474	-0.2000	0.074	
c34	1	2	-0.048		-0.088		-0.316	
c36	2	2	-0.007		-0.125		0.037	
c37	1	2	0.108		-0.457		0.381	
c38	2	2	0.037		-0.248		-0.087	
c39	2	2	-0.117		-0.184		-0.073	
c40	2	2	-0.098		0.015		0.047	
c41	2	2	-0.03		-0.315		-0.105	
c42	1	2	0.084		-0.294		0.037	
c45	2	2	-0.064		0.13		-0.12	
c47	2	2	-0.095		-0.381		-0.194	
c48	2	2	-0.179		0.129		-0.213	
c49	1	2	-0.235		-0.158		-0.251	
c50	1	2	-0.115					
c51	1	2	0.085		0		0.538	
c53	2	2	-0.165		-0.459		-0.052	
c57	2	2	-0.425		0.461		-0.319	
c58	1	2	-0.291		0.298		-0.218	
c59	1	2	0.082		-0.011		-0.028	
c60	1	2	-0.295		-0.164		-0.148	
c61	2	2	-0.026		0.169		0.229	
c63	1	2	-0.051		-0.256		0.678	
c64	1	2	0		-0.596		-0.158	
c65	2	2	-0.484		-0.065		-0.159	
c66	1	2	-0.504		0			

Table 3: Linear regression coefficients by individual calculated for the moderate stress experiment, exploration and memory test; B between seasons – latency to feed by 3 replicas calculated within each season, B overall – latency to feed by 6 repeats in total; Population 1 – Madrid, 2 – Cocentaina; Season 1 – autumn, 2 - spring





12 Chapter 5

“Dominance in blackcaps (*Sylvia atricapilla*) from three populations differing in migratory behaviour”

Bulaic M, Pulido F (unpubl. manuscript)





12.1 Introduction

Interactions between individuals through social relationships form dominance hierarchies within animal groups, where each individual can be characterized by its social rank (Chase, 1980). Dominance status is potentially an important component of fitness as it affects both survival and reproductive success. Dominant individuals may benefit from having priority access to better food resources (Hogstad, 1989; David *et al.*, 2011) and foraging sites with lower predators risk (Ekman, 1989) or have priority access to opposite sex, thus having greater success in mating (Otter and Ratcliffe, 1996).

Studies on hierarchy formation have focused on variation in physical characteristics of individuals. For example, in great tits fighting performance is affected by body size, weight, age and sex (Sandell and Smith, 1991; Lemel and Wallin, 1993), as well as by the size of their breast stripe (Sandell and Smith, 1991; Wilson, 1992). Apart from morphological traits, dominance status can be influenced by behavioural profile (i.e. personalities), which is important in the establishment of dominance hierarchies within social group, where some individuals are able to dominate others in competitive environments (Verbeek *et al.*, 1999; Reale *et al.*, 2000; Dingemanse and de Goede, 2004). For example, proactive female zebra finches (David *et al.*, 2011) or fast exploring great tits (Verbeek *et al.*, 1999) are more likely to be the dominant ones.

Different bird populations can vary in their migratory strategy. Populations may be completely migratory, partially migratory, where part of individuals migrates and others do not, or the entire population may be non-migratory. Factors responsible for partial migration probably include both genetic differences in migratory behaviour, as well as facultative response to environmental conditions (Gauthreaux, 1978; Lundberg, 1985; 1988, Chapman *et al.* 2011a, Pulido, 2011). Some age and sex classes are considered subordinate, e.g. juveniles and females are usually dominated by adults and males throughout the non-breeding season. In addition, it is this very fraction of the population, i.e. juveniles and females, being more migratory (Ketterson and Nolan Jr, 1983; Newton, 2008) which is expressed in higher probabilities of leaving the breeding areas in winter and in some cases migrating longer distances (Terrill, 1987; Hogstad, 1989). This phenomenon is known as differential migration (Cristol *et al.*, 1999). It has been hypothesized that differences in social dominance could be one of the causes of differential migration (Gauthreaux, 1978; Lundberg, 1985; Arizaga and Bairlein, 2011; Bai and Schmidt, 2012). “The dominance hypothesis” states that when the food is scarce and the food sources are occupied by dominants with priority access, subordinate individuals, as poor competitors, tend to accept the challenge of migration in order to avoid competition that could be more costly than the migration itself (Ketterson and Nolan Jr, 1979; Marra, 2000). This hypothesis has received support from several studies, e.g. Lundberg (Lundberg, 1985), found that juveniles and females of European blackbirds (*Turdus merula*) population lost their body fat and weight in mid-winter, while males and adults reached their peak, suggesting that females and juveniles are mostly the ones engaging in autumn migration. Rogers (Rogers *et al.*, 1989) studied two populations of dark eyed juncos (*Junco hyemalis*), that had crossed



different distances within autumn migration, and found that the ones with shorter distance were dominant over the others migrating further into their winter range, however, only in half of the pairwise encounters. Therefore, presumably the dominance status and migratory activity are correlated within individuals or populations, the question remains about the underlying mechanisms.

A genetic threshold model has been proposed for describing inheritance and evolution of migratory behaviour (Pulido *et al.*, 1996). The principle of this model is based on an underlying normally distributed variable (e.g. amount of proteins or hormones involved in migratory activity) that correlates with migratory activity. According to this model, individuals with values below the threshold do not express migratory activity and are classified as residents, while individuals with values above the threshold are migratory. An extension of the “threshold model of migration” was proposed in order to better describe the situation in natural populations, where the environmental factor is added, accounting for environmental variation as one of the determinants of migratory activity (Berthold, 1984; Adriaensen *et al.*, 1990; Pulido, 2011). This model predicts that individuals at the extremes of the distribution of the continuous variable are not affected by environmental factors, while those closer to the threshold can easily be tipped over to one side or the other, thereby readily changing migratory status depending on environmental conditions. Hence, changes in migratory activity depend not only on the genetically determined position of the threshold but also on environmental variables. Several environmental factors modifying the propensity to migrate or to remain sedentary have been proposed, such as food availability, temperature and dominance (Pulido, 2011). Yet their contribution in modifying the migration threshold has not yet been assessed. For this reason, we studied three Iberian blackcap (*Sylvia atricapilla*) populations (migratory, partially migratory and sedentary) using a “common garden” approach which allowed us to control for possible factors that could influence migratory activity and to determine if there is a correlation between migratory activity and individual dominance status, as determined in pairwise interactions. The aim of the present study was to test the “dominance hypothesis” in the framework of the environmental threshold model of migration. In order to shed additional light onto the possible correlations between dominance, migration and personality traits, we also tested personality profiles with behavioural experiments, as animal personalities have been shown to have an effect on dominance (Verbeek *et al.*, 1996; Dingemanse and de Goede, 2004). The combination of measurements of personality traits, dominance and migratory behaviour in the same individuals makes this study unique in its objectives and integrative approach.

12.2 Materials and methods

The study was conducted on male and female juvenile blackcaps (*Sylvia atricapilla*) from 3 Iberian populations selected for representing different migration strategies: Madrid (Pinilla del Valle 40°55'N, 3°49'W) as migratory (Tellería *et al.*, 2001), Cocentaina (Alicante) (38°44'N, 0°26'W) partially migratory (Morganti *et al.*, 2015) and Tarifa (Los Barrios 36°11'N, 5°36'W) as sedentary population (Tellería *et al.*,



2001). Juveniles were captured in 3 consecutive years when 2-3 months old: 2010 (N=33), 2011 (N=33) and 2012 (N=22), from the end of June until mid-July. Upon capture, each bird was assigned its own colour ring code, which facilitated distinguishing it from the others. After capture, birds were transported to our study facilities in a restricted area within the “Casa de Campo” Park in Madrid (40°25’N, 3°45’W). Birds were kept in individual cages (45x23x38 cm) with food and water *ad libitum*, under a natural photoperiodic regime based on a nearby Madrid location (weather station at Barajas, Madrid) and they had visual contact with individuals in neighbouring cages, and auditory contact with the rest. For dominance tests individuals were kept in the outdoor aviaries (3x2x2.3 m) supplied with 6 perches, a tree and undergrowth, to mimic their natural habitat. Food and water was given *ad libitum*.

Dominance experiments were conducted in spring, from April until the end of May, with spring migration at its finishing stage. This period was chosen because more individuals were available for testing once released to aviaries from the cages where they were kept in captivity since autumn. As the testing period was close to the breeding season and courtship and mating could have interfered with the experiments, we only tested birds of the same sex to determine dominance. In 2012, however, birds were also tested during the autumn season, for two weeks in mid-November. The experiments were filmed with hand cameras (Panasonic SDR-H85 and Sony DRC-SX65E) inconspicuously placed on the outside of the aviary.

After the experimental period in aviaries was finished at the end of May, all the individuals were released to their original capture sites and their colour rings were removed.

12.2.1 Dominance tested in pairs

For the determination of dominance, two individuals would be introduced simultaneously into a new, unfamiliar aviary, in order to avoid prior residency effects. Observations of social interactions were conducted the first time birds were introduced to the new aviary, and two more times with two days in between. We observed and scored their staged dyadic encounters for about 10 min, assigning within-pair dominance rank. The final dominance rank of each individual was determined by analysing typical dominance interactions, such as active and passive displacements, where the subordinate bird would repeatedly relinquished the perching site to the dominant one (Pravosudov *et al.*, 2003; Fox *et al.*, 2009). In that case, a score of one would be assigned to the approaching bird that would overtake the perching site, while the score of zero to the approaching individual that would fail to overtake the site and retreat. Mostly, the dominance relationship was established with little or no observed aggression towards the subordinate bird. The study was conducted on 43 pairs from all 3 cohorts in both seasons, with paired up individuals of the same sex, but from different populations to test for possible among-population differences. Populations were paired up in the following combinations: Madrid-Tarifa (16 pairs), Madrid-Cocentaina (19 pairs) and Cocentaina-Tarifa (8 pairs).



Although the paired birds were not in visual contact with one another in the aviaries before the test, they were still able to hear each other.

In addition to the previously described uni-sex tests, we conducted dominance tests in 9 mixed pairs of males and females of the same population to test for among-sex dominance relationships. These tests were conducted using the same protocol but done in winter (January) in order to avoid mating behaviour. By this comparison, we wanted to test if the males are dominant over females.

12.2.2 Dominance tested in groups of 4 birds

After the birds were kept in pairs for a week or so, groups of four were created by transferring two already existing (and tested) pairs into a new, unfamiliar aviary (20 in total). These groups again contained same sex individuals from different populations. Following the protocol used throughout this study, observations were repeated three times, with two days between observations and the same scoring system was used. The aim of this experiment was to observe how individuals with social ranks established within pairs would change dominance status when put into a larger group with one known and two unknown individuals – would the dominant individuals stay dominant, or degrade their rank due to stress after possibly losing some of the interactions (Verbeek *et al.*, 1999)?

12.2.3 Migratory activity

Nocturnal activity, restlessness or “Zugunruhe”, is a good indicator of migratory activity in birds in captivity (Berthold, 1996; Ramenofsky *et al.*, 2003), displayed by wing flapping and jumps. To determine the migratory activity of individuals, nocturnal activity was measured in 30 minute intervals during the lights out period for the individuals inside the cages that were connected to the recording system (for details see Chapter 1). The data of the onset and amount of migratory activity were used to link the migratory propensity with the individual’s dominance status in order to test our data for the validity of the “dominance hypothesis”, where the more migratory individuals should be less dominant.

12.2.4 “Personality” types

We have described some of the “personality” traits for our three test populations, by measuring latencies, i.e. the amount of time necessary for the individual to feed from the usual feeder and overcome the effect of the stressful or unfamiliar situation, within the moderate stress experiment, exploration and memory test (see Chapter 3 for details). With those data, we wanted to link individual’s “personality” type with its dominance status, as fast explorers were reported to have higher dominance score as well (Verbeek *et al.*, 1996; Dingemanse and de Goede, 2003).



12.2.5 Statistical analysis

Pairwise interaction dominance was calculated by dividing the number of wins by total number of initiated interactions (values from 0 to 1, see above), and weighted by the number of non-initiated interactions to correct for possible bias in dominance score for individuals with few initiated interactions, with no observed reversals within pairs (Pravosudov *et al.*, 2003). Total number of initiated interactions was considered as well as a possible factor in the final dominance score. Moreover, each individual was categorized as subordinate (final score=0) if the weighted dominance score was equal or below 0.4, and 1 or dominant (final score=1) if values were equal or above 0.6 (Verbeek *et al.*, 1999). The assigned dominance rank score within the group (from 1 to 4) was based on the difference in weighted dominance score within the group. Additionally, a dichotomous variable was created by pooling individuals with scores of 1 and 2 into the dominant category (score 1) and ones with 3 and 4 into the subordinate category (score 0). Linear regression coefficient was calculated from the weighted dominance score within pairs and weighted dominance score within groups.

A linear regression coefficient (B) was obtained from weighted dominance score for individuals in pairs and their average dominance score for groups (wins/individuals won against).

To test for the correlation between migratory activity and dominance, we used principle components of migratory activity (see Chapter 1, supplementary table 1). Personality traits were analysed as well by a principal component analysis (details in the Materials and methods of Chapter 3, supplementary table 2).

Variables not showing normal distributions were log transformed to achieve normality. Among-population and among-sex comparisons were analysed by the paired t-test, while correlations of personality and migratory traits were conducted by Pearson's correlation analysis.

12.3 Results

12.3.1 Among population effects

12.3.1.1 Comparison of Madrid and Tarifa populations in dominance score

The weighted dominance score, number of initiated interactions and number of won interactions showed no difference between the two populations in general or within same sexes of the two populations (Table 1). The number of initiated interactions (log) was positively correlated both with the number of won interactions (log) ($r=0.917$, $p<0.001$ (2-tailed), $N=32$) and the weighted dominance score ($r=0.382$, $p=0.031$ (2-tailed), $N=32$), while the number of non-initiated interactions (log) was negatively correlated with the weighted dominance score ($r=-0.382$, $p=0.031$ (2-tailed), $N=32$). In general, weight and tarsus size of the birds



did not show significant correlation with weighted dominance score. However, in females the correlation between tarsus length and dominance was close to significance ($r=0.651$, $p=0.058$ (2-tailed), $N=9$).

12.3.1.2 Comparison of Cocentaina and Tarifa populations in dominance score

Tarifa and Cocentaina birds showed a tendency towards differences in weighted dominance scores, where Tarifa individuals tended to have higher mean dominance scores ($t_{(7)}=-2.192$, $p=0.065$). There was no difference in weight between populations ($t_{(7)}=-1.562$, $p=0.162$), however, the tarsus size is bigger in individuals from Tarifa ($t_{(7)}=-4.243$, $p=0.004$), due to significant difference in females ($t_{(5)}=-3.909$, $p=0.011$) while males' sample size is too small.

Total number of initiated interactions was positively correlated with weighted dominance score ($r=0.563$, $p=0.023$ (2-tailed), $N=16$), and won interactions ($r=0.937$, $p<0.001$ (2-tailed), $N=16$), while the number of non-initiated interactions (log) was negatively correlated with the weighted dominance score ($r=-0.643$, $p=0.010$ (2-tailed), $N=15$). There was no correlation between dominance and weight, however, tarsus showed a positive correlation ($r=0.677$, $p=0.004$ (2-tailed), $N=16$). Regarding the difference between sexes, again, only in females dominance was correlated with tarsus ($r=0.682$, $p=0.015$ (2-tailed), $N=12$), while sample sizes in males were too small to test for this relation ($N=4$).

12.3.1.3 Comparison of Madrid and Cocentaina populations in dominance score

No differences were found in the comparison of the two populations (Table 1), neither in total nor within sexes. Tarsus size and weight as well did not differ between populations in general (tarsus $t_{(7)}=0.124$, $p=0.905$; weight $t_{(10)}=-0.974$, $p=0.353$) or within sexes (males tarsus $t_{(4)}=0.416$, $p=0.699$; weight $t_{(7)}=-1.273$, $p=0.244$; females' sample size too low).

The initiated interactions were positively correlated with the weighted dominance score, ($r=0.386$, $p=0.029$ (2-tailed), $N=32$), as well as with the number of won interactions in general ($r=0.936$, $p<0.001$ (2-tailed), $N=32$). No significant correlations were found between dominance, weight and tarsus variables.



	Madrid		Tarifa		N pairs	t (df)	p
	Mean	St. dev	Mean	St. dev			
Dominance score	0.55	0.30	0.45	0.30	16	0.674 (15)	0.510
<i>Dominance score males</i>	0.62	0.37	0.38	0.37	8	0.952 (7)	0.373
<i>Dominance score females</i>	0.48	0.21	0.52	0.21	8	-0.306 (7)	0.769
Won interactions (log)	1.15	0.53	0.91	0.44	16	1.455(15)	0.166
Total initiated interactions (log)	1.31	0.47	1.08	0.44	16	1.751 (15)	0.100
Weight	17.25	2.15	17.95	0.81	13	-1.184 (12)	0.259
<i>Weight in spring</i>	16.64	1.66	17.98	0.80	10	-2.727 (9)	0.023
Tarsus	20.77	0.46	21.18	0.38	5	-1.507 (4)	0.206

	Cocentaina		Tarifa		N pairs	t (df)	p
	Mean	St. dev	Mean	St. dev			
Dominance score	0.36	0.18	0.64	0.18	8	-2.192(7)	0.065
<i>Dominance score males</i>	0.46	0.21	0.54	0.21	2	-0.249(1)	0.845
<i>Dominance score females</i>	0.32	0.18	0.68	0.18	6	-2.415(5)	0.060
Won interactions (log)	1.02	0.37	1.29	0.44	8	-1.464(7)	0.187
Total initiated interactions	21.00	15.90	31.38	22.94	8	-1.081 (7)	0.315
Weight	16.76	1.76	17.43	1.36	8	-1.562(7)	0.162
<i>Weight in spring</i>	15.98	0.92	16.93	1.13	6	-4.094(5)	0.009
Tarsus	20.24	0.39	21.25	0.47	8	-4.243(7)	0.004
<i>Tarsus females</i>	20.18	0.39	21.18	0.45	6	-3.909(5)	0.011

	Madrid		Cocentaina		N pairs	t (df)	p
	Mean	St. dev	Mean	St. dev			
Dominance score	0.43	0.25	0.57	0.25	16	1.219(15)	0.242
<i>Dominance score males</i>	0.42	0.22	0.59	0.22	11	1.286(10)	0.228
<i>Dominance score females</i>	0.45	0.32	0.55	0.32	5	0.342(4)	0.736
Won interactions (log)	1.09	0.50	1.20	0.54	16	0.678(15)	0.508
Total initiated interactions (log)	1.29	0.50	1.37	0.53	16	0.598(15)	0.559
Weight	16.69	1.09	17.19	1.73	11	-0.974(10)	0.353
Tarsus	20.87	0.46	20.82	1.03	8	0.124(7)	0.905

Table 1: Paired t-tests comparisons of the Madrid, Cocentaina and Tarifa populations' weighted dominance score, number of won and started interactions, as well as their weight and tarsus sizes. Main comparison studied variables are marked in bold, and differences within sexes in italic. Main variables were tested within sexes as well, and only significant results are given in the table; $p < 0.05$.

12.3.2 Comparison of male and female individuals during the winter season

Males did not show higher mean weighted dominance scores ($t_{(8)}=0.092$, $p=0.929$), number of initiated interactions ($t_{(8)}=-1.067$, $p=0.317$) or won interactions ($t_{(8)}=-0.635$, $p=0.543$) than females. The sample size, however, was too low for comparison by populations. Weight has not been found to have any significant effect ($t_{(7)}=-1.209$, $p=0.266$) between sexes.

12.3.3 Change of dominance score from pairs to groups

Significant difference in its mean was showed for groups of subordinates and dominants from the pairwise encounters, where subordinates have positive mean of B (mean 0.165), while it is negative for dominants (mean -0.304) ($t_{(21)}=4.788$, $p<0.001$).

The final rank from 1 to 4 assigned to each of the groups' individuals was compared by crosstabulation to their previous pairwise scores without any significant change of the result ($\chi_{(3)}=0.521$, $p=0.914$). Same absence of significant result was found when analysis was conducted separately for sexes (males $\chi_{(3)}=2.631$, $p=0.452$; females ($\chi_{(3)}=4.997$, $p=0.172$). Additionally, the ranks from 1 to 4 were summed up to only two: 0



and 1 (1,2=1; 3,4=0). This analysis, as well, provided no evidence for an association between the dominance status in the groups of 2 and in the groups of 4 ($\chi_{(1)}=0.095$, $p=0.758$, Fisher's exact test $p=0.786$; Table 2).

		Rank within the group (0,1)		Total
		0	1	
Pairwise interaction score	0	14	17	31
	1	13	11	24
Total		27	28	55

Table 2: Relation between individuals' dominance status within pairs (Pairwise interactions score; 0 and 1) and its dominance rank within the group of four individuals (Rank within the group), (0-subordinate, 1-dominant).

Dataset split by sex, as well, did not show statistically evidence for a maintenance of dominance status (see Table 3; males $\chi_{(1)}=0.694$, $p=0.405$, Fisher's exact test (2-sided) $p=0.442$; females ($\chi_{(1)}=1.448$, $p=0.229$, Fisher's exact test (2-sided) $p=0.276$). However, the exchange of dominance status between pairs and groups appears to be more evident within females where 66.7% of subordinate females within pairs find themselves within the dominant group of individuals in aviaries, while 69.2% of previously dominant belong now to the subordinate class.

		Rank within the group (0,1)		Total
		0	1	
Males	Pairwise interaction score	0	9	7
		1	4	7
	Total	13	14	27
Females	Pairwise interaction score	0	5	10
		1	9	4
	Total	14	14	28

Table 3: Relation between within-pairs dominance status (pairwise interactions score) and dominance ranks within the group of four individuals (rank within the group), split for males and females (0-subordinate, 1-dominant).

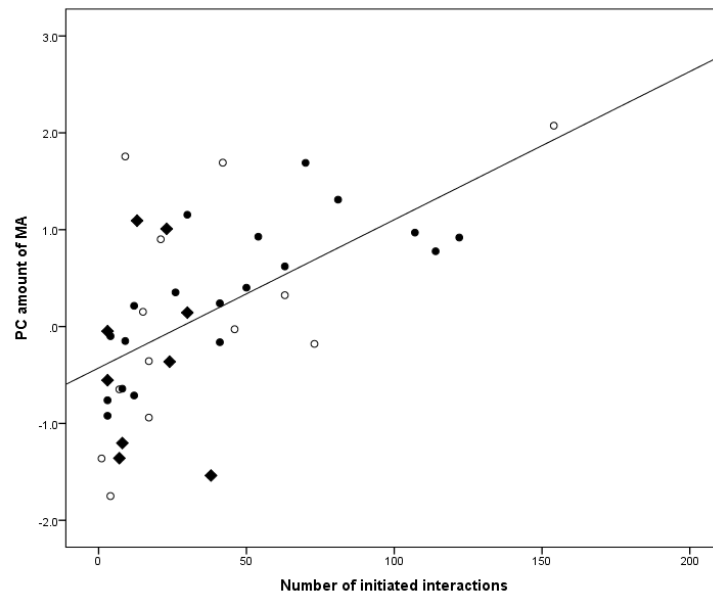
There was no population effect in the change of ranks from pairs to groups (Madrid $\chi_{(1)}=0.020$, $p=0.888$, Fisher's exact test (2-sided) $p=0.100$; Cocentaina $\chi_{(1)}=0.524$, $p=0.469$, Fisher's exact test $p=0.667$; Tarifa $\chi_{(1)}=0.625$, $p=0.429$, Fisher's exact test (2-sided) $p=0.571$).

12.3.4 Correlation of dominance score with migratory activity

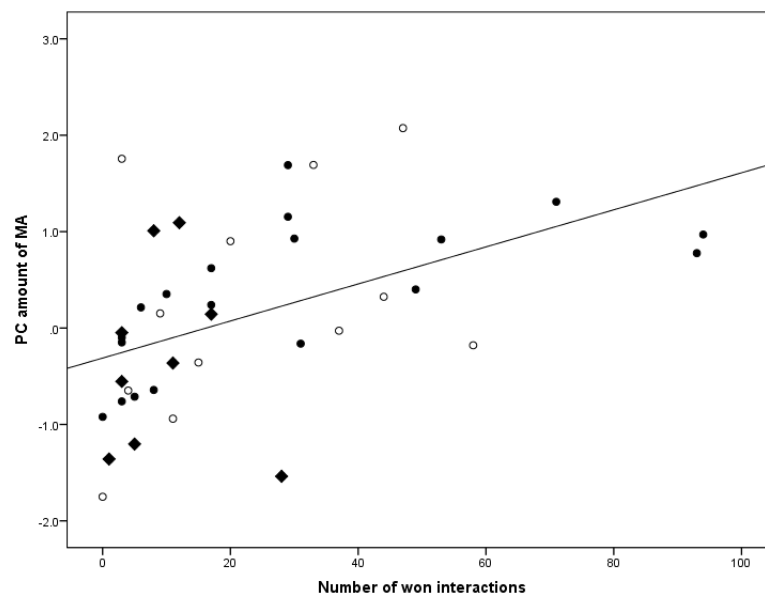
No significant correlations were found between PC's for the onset of migratory activity and the weighted dominance score or number of initiated and won interactions. The amount of migratory activity showed significant interactions where PC amount of activity (PC1) is positively correlated with number of initiated ($r=0.585$, $p<0.001$, $N=41$) and won interactions ($r=0.482$, $p=0.001$, $N=41$), while tendency is present for the correlation with weighted dominance score ($r=0.271$, $p=0.086$, $N=41$). When the analysis is done by populations, we find positive correlations between amount (PC1) and number of initiated interactions within Madrid population ($r=0.558$, $p=0.048$, $N=13$) and the partially migratory Cocentaina population (PC1 amount



of activity by number of won interactions $r=0.636$, $p=0.003$, $N=19$; PC1 amount of activity by number of initiated interactions $r=0.736$, $p<0.001$, $N=19$), but no significant correlations within Tarifa.



Graph 1: Scatterplot of the number of initiated reactions within pairwise tests and the principal component representing the amount of migratory activity ($B=0.015$, $p=0.001$); populations are marked with different symbols: Madrid - empty circles, Cocentaina - filled circles, Tarifa - filled squares.



Graph 2: Scatterplot between number of won reactions within pairwise tests and principal component representing the amount of migratory activity ($B=0.019$, $p=0.001$); populations are marked with different symbols: Madrid - empty circles, Cocentaina - filled circles, Tarifa - filled squares.



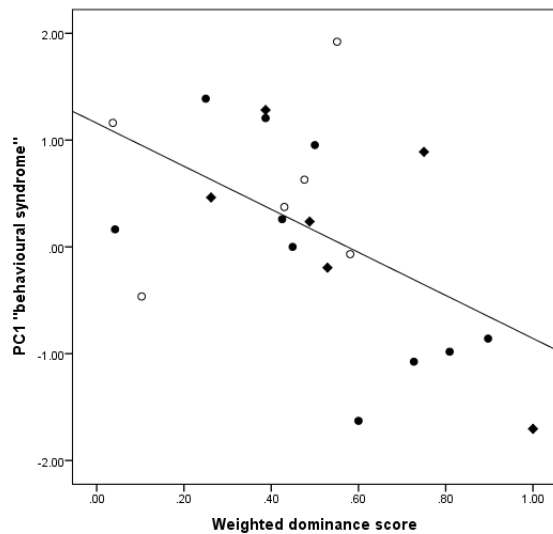
12.3.5 Correlation of dominance score with personality traits

We found a negative correlation between PC1 “behavioural syndrome” (reflecting variation of the latency variables measured in the exploration and memory tests) and weighted dominance score; and positive correlation between PC2 “behavioural syndrome” (variance in latencies of the moderate stress experiment) and the number of initiated and won interactions (see Chapter 3 on details for the two PC’s) (Table 3).

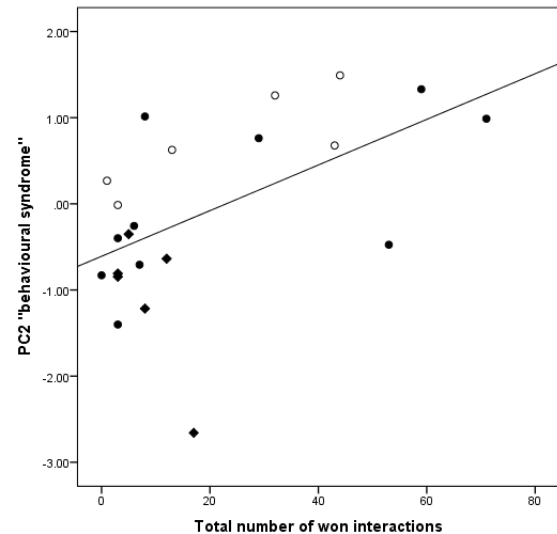
	PC1 "behavioural syndrome"	PC2 "behavioural syndrome"
Mean weighted dominance score	-0.520*	0.134
Total number of initiated interactions	0.192	0.480*
Total number of won interactions	-0.010	0.546**

Table 3: Pearson’s correlation coefficients between mean weighted dominance score, number of initiated and number of won interactions and principal components (PC) extracted from all variables defining personality traits within the moderate stress , exploration and memory test experiment; * Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

Looking into differences between losers and winners for all the personality variables, individuals defined as dominant, based on their latency values in each experiment, proved to have significantly shorter latencies in approaching the novel food plate in the exploration test ($t_{(12)}=2.734$, $p=0.017$, $N=14$), and a tendency towards shorter memory test latencies when approaching the covered food plate ($t_{(9)}=2.071$, $p=0.068$, $N=10$). No difference among dominant and subordinate individuals was found for moderate stress experiment. The same analysis ran on the two principal components obtained from the all originally extracted personality variables (behavioural syndrome analyses, see details in Chapter 3) showed only non-significant, but close to differences between the PC presenting the variables of the exploration and memory tests for the dominant (mean=-0.40) and subordinate (mean=0.66), which, however, were close to significance ($t_{(8)}=2.161$, $p=0.063$, $N=9$).



Graph 3: Scatterplot between weighted dominance score obtained from pairwise interactions tests and principal component (PC1) representing the “behavioural syndrome” (all the personality tests described variables) ($B=-2.015$, $p=0.013$); populations are marked with different symbols: Madrid - empty circles, Cocentaina - filled circles, Tarifa - filled squares.



Graph 4: Scatterplot between total number of won reactions within pairwise interactions tests and principal component (PC2) representing the “behavioural syndrome” (all the personality tests described variables) ($B=0.026$, $p=0.009$); populations are marked with different symbols: Madrid - empty circles, Cocentaina - filled circles, Tarifa - filled squares.

12.4 Discussion

Here, we examined possible differences in dominance status between a migratory (Madrid), a partially migratory (Cocentaina) and a sedentary population (Tarifa), as well as correlations between dominance status, level of migratory activity and personality traits. The population comparisons between Madrid and Tarifa and Madrid and Cocentaina showed no difference in weighted dominance score, or number of won or initiated interactions. The Tarifa-Cocentaina comparison, as well, did not provide any evidence for among-population differences in dominance, although a tendency was present for Tarifa individuals being dominant over Cocentaina (Tarifa-6 defined as dominant individuals, Cocentaina-2).

Positive correlations between the number of initiated and number of won interactions with the weighted dominance score were found within all three population comparisons. Moreover, we found a negative correlation between number of non-initiated interactions and weighted dominance in two out of three population comparisons, indicating that the dominant individuals were the ones initiating interactions as it was previously found in juvenile great tits (Verbeek *et al.*, 1996; Verbeek *et al.*, 1999).

Both in comparisons of Madrid and Cocentaina with Tarifa population, a positive correlation was found between tarsus size and weighted dominance score within females. As the tarsus size is one of the indicators of the body size of an individual (Freeman and Jackson, 1990; Senar and Pascual, 1997), it confirms



previously found results that body size and condition have an effect on dominance (Lindström *et al.*, 1990), found for example in two studies of importance of the size on dominance in bluethroats (*Luscinia svecica*) and wheatears (*Oenanthe oenanthe*) in the wild (Lindström *et al.*, 1990; Dierschke *et al.*, 2005). The absence of the correlation within males might be due to the low sample size.

No differences in dominance between sexes were found in winter, possibly due to a general absence of sexual size dimorphism in blackcaps (Pérez-Tris and Tellería, 2002; Catry *et al.*, 2006). The situation might be different within different seasons or while migratory active, like in autumn. In northern wheatears (*Oenanthe oenanthe*) where there is a significant sex-effect on dominance, males being dominant over females, the effect was absent in winter (Arizaga and Bairlein, 2011). Moreover, in spring, males are more dominant as they are usually arriving earlier than females in order to establish their breeding territories (Rubolini *et al.*, 2004; Dierschke *et al.*, 2005) with much higher testosterone levels than females due to gonadal maturation (Dawson, 1983; Schwabl and Kriner, 1991; Dawson *et al.*, 2001).

The amount of migratory activity was positively correlated with number of initiated and won interactions, and we also found a tendency for a positive correlation between weighted dominance score and migratory activity. As both the numbers of won and initiated interactions are strongly positively correlated with dominance score, we can presume that social dominance is truly one of the important factors related to migratory activity. These results suggest that in Iberian blackcaps higher dominance scores are found in more migratory individuals. However, a study of a partially migratory population from Cocentaina found that, during winter, although smaller in size, the residents were dominant over the migratory overwintering birds (Morganti *et al.*, unpublished manuscript). Our results are opposite to the “dominance hypothesis” (Gauthreaux Jr, 1978), which predicts that the more dominant individuals occupy the best food sources; thus do not have to leave their breeding territories in winter, suggesting some other limiting factor involved in our system. That factor could not be the food availability, as our test birds had *ad libitum* food, creating a situation possibly analogous with bird populations in tropical areas, where food is not the main migration causing factor, and more dominant birds were the ones more likely to migrate (Boyle, 2008; Jahn *et al.*, 2010). In fact, in our study of migratory activity (Chapter 1), we found no evident population effect in the amount of migratory activity, thus, we cannot make presumptions that the less migratory birds are the ones coming from the Tarifa population, and that Tarifa, as a sedentary population, has lower dominance score. As predicted by the extended “threshold model” (Pulido, 2011), keeping the birds at the Madrid’s latitude might have been the cause for expression of migratory activity in similar levels within all three populations, confounding in that way the true dominance-migratory strategy relationship for our species.

12.4.1 Dominance and personality

Within all the three population comparisons, we found positive correlations between weighted dominance score for pairwise encounters, number of initiated and number of won interactions, which suggest



that the more dominant individuals are as well more active. We also compared the mean weighted dominance score of the pairwise encounters of each individual with latencies in the personality experiments. Individuals with higher dominance score were more affected by the short term moderate stress situation (PC2 “behavioural syndrome”), where the usual feeder was removed for a short period of time. However, latencies in the exploration and memory tests (PC1 “behavioural syndrome”) were lower for the more dominant individuals, a result that could maybe reflect lower neophobia in a novel situation. Dominant individuals proved to have shorter latency to approach the unfamiliar plate (tendency in memory test)(Mettke-Hofmann *et al.*, 2002), their shorter approaching time to novel object implying that dominant individuals tend to be fast explorers (Verbeek *et al.*, 1996; Boogert *et al.*, 2006; David *et al.*, 2011). This is not an isolated case, as the study of great tits showed that fast exploring territorial males were also dominant over slow exploring ones (Dingemanse and de Goede, 2004). We can presume that within our birds territoriality was present as well, as they were kept in pairs within aviaries, with no space for non-territorial wandering individuals. Results on these questions from other studies are contradictory. For example, if dominant individuals are monopolizing the food source, subordinate individuals may be forced to look for other resources, being faster explorers and less neophobic in search for food, in the effort to level their survival chances with the dominant ones (Fox *et al.*, 2009). Another characteristic of dominant and fast exploring individuals was rigidity and lower flexibility in adjusting their behaviour in a changed situation, displayed by tendency towards stronger expressed old habits within the exploration and memory test and higher stress level caused by the change in everyday routine when the usual feeder was removed for 20-30 min (Benus *et al.*, 1991; Verbeek *et al.*, 1999).

As mentioned, our results imply that the dominant blackcaps tend to be faster explorers. One of the examples for this correlation is in great tits (*Parus major*) study, where faster exploring individuals generally become dominant in staged dyadic encounters (Verbeek *et al.*, 1996). However, the situation changed once they found themselves within groups in aviaries where they became subordinate to slow exploring individuals (Verbeek *et al.*, 1999). Here, another factor was responsible for the social hierarchy, and that is the individual’s ability to cope with stress, where slow explorers were recovering faster from stressful situations (Dingemanse and de Goede, 2004) in semi natural situations, like aviaries, while dominant ones were being more affected by defeat. Our individuals have displayed the same change in dominance status from pairs to social groups, where the individuals that were dominant in pairwise encounters, loose their dominant position on the hierarchy scale (negative linear regression coefficient for the dominant individuals within pairs), caused probably by poor stress coping due to lost interactions (Carere *et al.*, 2001; Carere *et al.*, 2003; Verbeek, 1998).

We believe our study’s importance lies in questioning possible correlations between dominance, migration and personality traits, where the combination of their measurements within the same individuals makes it unique in its objectives and integrative approach. However, one of the strongest limitations of this experimental set-up is that we have studied only one population from each of the migration strategies. As a



consequence, we cannot tell apart with certainty the differences in dominance behaviour that are originating from different types of migratory behaviour, and differences due to other among-population dissimilarities (e.g. latitude, density, etc.). As well, pairwise comparison might not be able to find more statistically significant effects due to low sample size or the conditions that they were reared in, as they were taken in young age and the keeping conditions of the “common garden” experiment might have altered the natural populations’ phenology; housing on the Madrid latitude could have influenced their migratory habits and make them all more similar to migratory population. Thus, aside from genetic conditioning of the migratory, personality and dominance traits, the environment may play a significant role in the development and expression of those traits. Hence, future studies should stress on including more than one population per migratory strategy category, as well as controlling for the environmental influence on the expression of the studied phenological traits, by interchanging populations and their corresponding environmental conditions.



12.5 Bibliography

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12.6 Supplementary material

	Onset			Amount	
	PC	PC act1	PC act2	PC	PC act2
% of variance	74.773	80.266	93.4	67.754	23.117
Eigenvalue	3.739	2.408	1.869	5.42	1.849
Factor loadings					
Onset of MA 5 days criteria	0.828	0.816			
Onset of MA 5 days criteria higher activity	0.884		0.947		
Onset >3 intervals of activity in the central part of the night	0.940	0.948			
Onset >4 intervals of activity the central part of the night (act 2)	0.830		0.947		
Onset >3 intervals of activity, independent of continuity	0.836	0.918			
Average MA corrected for the maximum number of days of activity				0.866	-0.441
Sum of 30min activity corrected for days with more than >3 intervals and missing data				0.841	-0.48
Average MA corrected for the maximum number of days of higher activity				0.838	0.449
Sum of 30min activity corrected for days with more than >3 intervals and missing data (act 2)				0.758	0.569
Average MA corrected for the maximum number of days of activity (filled gaps)				0.87	-0.449
Sum of 30min activity corrected for days with more than >3 intervals and missing data (filled gaps)				0.836	-0.461
Sum of 30min activity corrected for days with more than >3 intervals and missing data (act 2, filled gaps)				0.747	0.567
Average MA corrected for the maximum number of days of higher activity (filled gaps)				0.818	0.404

Table 1: Summary of the principal component factor analysis of the onset of migratory activity and amount of the migratory activity for the autumn seasons only of the 2010-2013 period

	Moderate stress	Exploration test			Memory test		
	PC	PC1	PC2	PC3	PC1	PC2	PC3
% of variance	74.383	45.564	19.226	17.841	50.965	18.074	14.48
Eigenvalue	2.232	3.189	1.346	1.249	3.568	1.265	1.014
Factor loadings							
<i>Latency to feed</i>	0.961	0.965	0.002	0.191	0.968	0.087	0.087
<i>Latency to approach the usual/new/covered plate</i>	0.914	0.963	-0.076	0.144	0.967	-0.009	0.102
<i>Total time spend around the usual feeder</i>	0.686	0.718	-0.123	-0.515	0.914	0.066	-0.300
<i>Number of visits to the usual feeder</i>		0.649	0.168	-0.609	0.790	-0.238	-0.393
<i>Latency to approach the usual feeder</i>		0.603	-0.003	0.742	0.484	0.033	0.828
<i>Number of visits to the new/covered feeder</i>		0.154	0.802	0.003	0.037	0.777	0.096
<i>Total time spent around the new/covered feeder</i>		-0.088	0.808	0.062	0.009	0.769	-0.237

Table 2: Summary of the principal component factor analysis of moderate stress experiment, exploration and memory tests



13 Resumen

"Variación en la expresión de la actividad migratoria en la curruca capirotada (*Sylvia atricapilla*); efectos de origen, condición ambiental, dominancia y personalidad"





13.1 Introducción/Marco teórico

La migración es una respuesta a cambios estacionales del clima generando desplazamientos periódicos entre hábitats de cría y de invernada, permitiendo así el uso temporal de los recursos disponibles. La migración implica unos costes energéticos muy elevados, un aumento de la depredación potencial, variaciones ambientales y una disponibilidad de alimento impredecible a lo largo de la ruta migratoria; por lo que es una de las actividades más desafiantes de su ciclo vital. A pesar de ello, los beneficios de la migración compensan sus costes. La migración está programada genéticamente, siendo relativamente constante en su momento, distancia y dirección. Por otro lado, ambiente juega un papel predominante en algunas poblaciones, pudiendo modificar el comportamiento migratorio de una estrategia parcial o facultativa a un modo de vida sedentario.

Con el fin de describir el origen y evolución del comportamiento migratorio en aves, se ha propuesto un “modelo de umbral” genético para determinar si un ave es migrante o sedentaria. Dentro de una variable continua (p.ej. la concentración de proteínas u hormonas), este modelo asume que existe una actividad migratoria subyacente implicada en su expresión génica. Este umbral divide cada variable en categorías dicotómicas que definen el fenotipo de un individuo. Los ejemplares sin actividad migratoria muestran valores por debajo de este umbral, siendo clasificados como sedentarios, mientras que los ejemplares migrantes muestran valores por encima del umbral definido. Los cambios de estrategia vital no dependen únicamente de la posición del umbral determinado genéticamente sino también de las variables ambientales, por lo que dichas variaciones deben ser añadidas al modelo. Este modelo de umbral ambiental predice que el carácter migratorio de los individuos situados en los extremos de distribución no se encuentra afectado por los factores ambientales, mientras que aquellos más próximos al umbral pueden más fácilmente cambiar su estrategia migratoria.

Los diferentes ambientes se diferencian en el grado en el cual las condiciones varían con el tiempo. Por lo tanto, distintos tipos de comportamiento, como es el caso de las “personalidades”, podrían ser ventajosos en algunas poblaciones y desventajosos en otras. Actualmente es poco conocido si las diferentes estrategias migrador/sedentario pueden tener una influencia en los rasgos de “personalidad”, y si es así, como se enfrentan estas poblaciones a los cambios que requieren una respuesta específica.

Aparte de las diferencias genéticas y las distintas respuestas comportamentales frente a un entorno variable, las condiciones sociales intra e interpoblacionales pueden estar correlacionadas con las estrategias de migración. En hábitats donde la fuente de alimento es escasa, los individuos dominantes podrían desplazar a los ejemplares subordinados, más propensos a asumir los costes que conlleva la migración a fin de evitar la competencia intraespecífica. La confirmación de este hecho se puede encontrar a nivel intrapoblacional dentro del área ibérica mediterránea, donde las poblaciones residentes muestran un carácter dominante durante de la temporada no reproductiva.



13.2 Objetivos y resultados

El objetivo de este estudio es identificar y evaluar la importancia de los factores ambientales implicados en el comportamiento migratorio, así como los efectos de los diferentes tipos de comportamiento y su dominancia sobre la actividad migratoria. Para ello, se ha investigado el comportamiento migratorio de tres poblaciones Ibéricas de curruca capirotada (*Sylvia atricapilla*), que difieren en su comportamiento migratorio. Estas poblaciones presentan las tres distribuciones principales de la estrategia migratoria del "modelo de umbral" empleado: comportamiento migratorio, parcialmente migratorio y sedentario. Los efectos de la población en los rasgos de personalidad y dominancia social se estudiaron con el objetivo de evaluar la importancia de dichos factores sobre los diferentes comportamientos migratorios. Para cada individuo, la combinación de los parámetros relacionados con la personalidad, el comportamiento dominante-subordinado y el tipo de estrategia migratoria permiten obtener un enfoque global de los procesos implicados en el comportamiento migratorio.

Para llevar a cabo estos objetivos, el estudio se dividió en cinco partes, investigando en cada una de ellas un aspecto comportamental diferente.

En primer lugar, se estudiaron las diferencias en el inicio del periodo de migración y cantidad de actividad migratoria en cautividad durante el otoño. No fueron observadas diferencias significativas en la cantidad de actividad migratoria entre las poblaciones comparadas. Sin embargo, en relación al tiempo de inicio del periodo de migración, la población migradora adelantó temporalmente su actividad migratoria.

En segundo lugar, se comparó la actividad migradora en otoño dentro y entre dos tipos de ambientes distintos: las jaulas, representando un ambiente artificial, y aviarios al aire libre, que representan un medio más natural. Se observó que el mantenimiento de las aves en los aviarios sólo tuvo un pequeño efecto sobre la expresión de su comportamiento migratorio. En los aviarios, las curruca comenzaron su actividad migratoria significativamente más temprano que aquellos ejemplares situados en jaulas. Por otro lado, no se observó ningún efecto sobre la cantidad de actividad migradora. En estos experimentos se comprobó la posible influencia de las variables ambientales como la temperatura y condiciones meteorológicas sobre la inquietud migratoria. Se observó que la actividad de los individuos en los aviarios se encontró afectada por las condiciones meteorológicas, ya que el mal tiempo causó una reducción de la actividad migradora.

Para cada una de las tres poblaciones de estudio, en el tercer capítulo se describieron los rasgos de "personalidades" a nivel de individuo mediante la medición de sus latencias a distintas variaciones ambientales experimentales. El objetivo de este capítulo fue explorar la correlación entre el tipo del comportamiento y su predisposición migratoria. Los resultados sugirieron que la población sedentaria tiene más éxito en hacer frente al estrés moderado frente a la población migratoria que mostró latencias más largas.



Además, al ser modificada la ubicación del comedero dentro del aviario, los ejemplares sedentarios mostraron una menor flexibilidad a esta modificación ambiental, mostrando sus comportamientos de alimentación habituales y, no se adaptaron, o lo hicieron despacio, al ser modificadas las condiciones experimentales iniciales. En relación a las tasas de exploración, los análisis no mostraron diferencias estadísticamente significativas entre las tres poblaciones de estudio. Sin embargo, los resultados de la prueba de memoria sugirieron que la población sedentaria tiende a tener latencias de exploración/neofobia inferiores.

En el cuarto capítulo se analizó la relación entre el comportamiento migratorio y la capacidad para la memoria a largo plazo. Para ello, los experimentos comportamentales fueron repetidos tres veces, tanto en otoño como en la primavera siguiente. El acortamiento, o el mantenimiento, de las latencias observado desde el final de la temporada de otoño a la primera prueba en la primavera indicó que el comportamiento aprendido en las tres pruebas en otoño se mantuvo al menos durante un período de cuatro meses. No se encontraron diferencias interpoblacionales en el comportamiento aprendido.

Finalmente, en el quinto capítulo se determinó la relación entre la predisposición migratoria y el estatus de dominancia. Se observó experimentalmente en encuentros por pares el establecimiento de una jerarquía social entre curruca pertenecientes a diferentes poblaciones. En contra de lo supuesto inicialmente, la población migratoria mostró la tendencia para un mayor número de interacciones correlacionadas positivamente con un comportamiento dominante. Por otro lado, la población parcialmente migratoria mostró una posición subordinada a la población sedentaria. Finalmente, el análisis de la correlación entre el carácter dominante y los tipos de personalidad dio como resultado que los ejemplares con mayor dominancia mostraron unas latencias más cortas al aproximarse a un comedero desconocido. Además, los individuos dominantes mostraron con más fuerza viejos hábitos, volviendo frecuentemente a su comedero vacío, aún existiendo un comedero nuevo con alimento. Estos resultados sugieren que los individuos dominantes son exploradores rápidos, mostrando periodos de latencia más cortos, pero con menos flexibilidad a ajustar su comportamiento al modificar la ubicación de un recurso. Aparte de la exploración, otro de los factores responsables de la jerarquía social es la capacidad del individuo para hacer frente al estrés. Los individuos clasificados como dominantes en la comparación por pares, tendieron a perder su posición jerárquica al encontrarse dentro de un grupo social más grande, probablemente por un mal afrontamiento del estrés y una lenta recuperación causada por interacciones perdidas con sus conespecíficos.

13.3 Conclusiones

Los resultados obtenidos en esta Tesis han demostrado que, al contrario de lo observado en estudios anteriores, todas las poblaciones analizadas de curruca capirotada muestran inquietud migratoria. Las poblaciones de estudio no mostraron diferencias en sus estrategias migratorias a nivel genético, posiblemente causado por su proximidad geográfica, por lo que la elección de una u otra estrategia parece ser inducida por las condiciones ambientales. Estas poblaciones se encuentran próximas al umbral de migración, siendo



especialmente sensibles a las condiciones ambientales. De hecho, las diferencias interpoblacionales observadas al modificar las variables ambientales refuerzan esta hipótesis de sensibilidad ambiental. Los ejemplares pertenecientes a la población sedentaria mostraron el mayor modificación de la actividad migratoria al ser expuestos a diferentes ambientes, mientras que la población migratoria mostró unos cambios comportamentales menores.

La ausencia de diferencias poblacionales parece ser el resultado del alojamiento de las tres poblaciones en el mismo área, con condiciones experimentales para poblaciones migratorias (Madrid), las aves respondiendo a estas condiciones ambientales. Como consecuencia, el umbral de migración podría haberse desplazado hacia la izquierda, explicando la expresión de la conducta migratoria observada dentro de las tres poblaciones de estudio.

Los resultados obtenidos no han permitido confirmar el mayor carácter exploratorio de los ejemplares residentes, mostrando la población sedentaria un comportamiento más rígido al ser modificada la ubicación de los comederos. Por otra parte, las poblaciones migratoria y parcialmente migratoria mostraron una mayor flexibilidad comportamental, desechando el comedero más habitual y vacío más rápidamente. Durante la migración, los individuos poseen de un tiempo limitado para buscar alimento en las áreas de parada, por lo que un comportamiento más flexible podría ser una ventaja. Además, se observó que el comportamiento aprendido es mantenido por un período de cuatro meses, lo que indica la existencia en estas poblaciones de una memoria a largo plazo.

La población migratoria mostró una mayor tendencia a presentar individuos marcados como dominantes, a diferencia de población sedentaria y parcialmente migratoria, sugiriendo que el comportamiento dominante no determina la estrategia migratoria. La diferencia de dominancia entre las poblaciones migrante y residente podría ser propiciada con el fin de obtener una mayor acumulación de reservas para la temporada migratoria en otoño o por una mayor motivación por llegar temprano a las áreas de cría y establecer su territorio. La población parcialmente migratoria tiende a situarse en una posición subordinada frente a la población sedentaria, explicado por la hipótesis de un mayor comportamiento dominante en los individuos sedentarios más grandes. Los ejemplares con puntuaciones de dominancia más elevadas tendían a ser exploradores más rápidos, mostrando además una menor flexibilidad de adaptación en su comportamiento al modificar la ubicación del alimento, confiando más en su experiencia previa.

De la misma manera, la capacidad de los ejemplares para afrontar el estrés parece ser otro de los factores responsables de la jerarquía social en la curruca capirotada. Este hecho se hizo evidente por el cambio de rol de los ejemplares dominantes al ser introducidos en grupos más grande, pasando de dominantes a subordinados.

En la presente Tesis se ha confirmado que las diferencias interpoblacionales observadas en la migración son principalmente generadas por las diferencias ambientales y no, como se ha sugerido previamente, por



diferencias genéticas. Las condiciones ambientales dadas experimentalmente indujeron un comportamiento migratorio en las tres poblaciones de estudio, aunque varias de las variables ambientales estudiadas (alimento, densidad, competencia), que previamente habían sido consideradas como factores importantes en el control de la migración, propiciaron el comportamiento sedentario. Por lo tanto y para finalizar, las variantes ambientales como la disponibilidad de alimento, la densidad, la dominancia y los tipos de personalidad no parecen ser los principales determinantes de la variación interpoblacional migratoria observada en las poblaciones ibéricas de curruca capirotada. Por ello sería necesario realizar nuevos experimentos adicionales que incluyan otras variantes ambientales como la presión atmosférica y los campos magnéticos, así como su influencia sobre las variantes analizadas en esta Tesis.