

Madarak vonuláshoz való morfológiai adaptációi

Morphological adaptations to migration in birds

Egyetemi doktori (PhD) értekezés

VINCZE ORSOLYA

Témavezető:

Prof. Barta Zoltán

egyetemi tanár

Konzulens:

Dr. Pap Péter László

egyetemi docens

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Témavezetők:

Témavezető neve Prof. Barta Zoltán

Konzulens neve Dr. Pap Péter László

A doktori szigorlati bizottság:

elnök: Dr. Magura Tibor

tagok: Dr. Herczeg Gábor

Dr. Pénzes Zsolt

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Contents

Acknowledgements	1
CHAPTER I	3
General introduction	3
<i>Why do birds migrate?</i>	7
<i>Costs of migratory behavior and ways to minimise them</i>	9
Objectives and major results	20
Paper I	20
Paper II	21
Paper III	23
Paper IV	24
General discussion	26
<i>Adaptations to migration</i>	26
Concluding remarks	33
References	34
CHAPTER II	45
List of publications related to the dissertation	45
Personal contribution to publications included in the thesis	46
Certified list of publications	47
Paper I	51
<i>C.I. Vágási, P.L. Pap, O. Vincze, G. Osváth, J. Erritzøe, A.P. Møller</i> <i>2016 Morphological adaptation to migration in birds. Evolutionary</i> <i>Biology, 43, 48-59.</i>	
Paper II	63
<i>O. Vincze 2016 Light enough to travel or wise enough to stay? Brain</i> <i>size evolution and migratory behaviour in birds. Evolution 70, 2123–</i> <i>2133.</i>	

<i>Paper III</i>	74
<i>O. Vincze, C.I. Vágási, P.L. Pap, G. Osváth, A.P. Møller 2015 Brain regions associated with visual cues are important for bird migration. Biology Letters, 11, 20150678.</i>	
<i>Paper IV</i>	78
<i>O. Vincze, C.I. Vágási, P.L. Pap, C. Palmer, G. Dyke, A.P. Møller Migration distance and wing morphology predict accumulated fuel loads in bird. Manuscript.</i>	

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CHAPTER I

In this chapter I introduce the general topic and scientific background of my thesis. I define the major questions and objectives of my studies, provide an outline of the work conducted and I shortly discuss my major findings and results.

General introduction

Every year, millions of birds take on wings and travel large distances of up to several thousand kilometers to reach their wintering and then couple of months later their breeding grounds (Newton 2008). Due to the fascinating number of birds involved, the eye-catching flock movements and the astonishing synchrony of departure from and arrival to the breeding or wintering grounds avian migration has long been attracting the interest of both scientists and non-specialists. Why do birds migrate? Where do they go? How do they find their ways? Which morphological and physiological adaptations make such long distance migration possible? How can birds sustain flight for days or for over a week with no access to water, food or land to rest? These questions are only some of those that people were eager to answer for centuries. Despite the tremendous scientific efforts even these fundamental question often lack exhaustive answers and therefore remain to be further explored (Alerstam 2006, Wink 2006, Newton 2008).

Migration is the active directional movement of animals over relatively long-distances, occurring annually, usually on a seasonal basis, triggered by climate or resource fluctuations and involves a relatively synchronous movement of animals over space (Rappole 2013). Migratory behaviour is widespread across all animals and it developed along multiple independent evolutionary paths in distant animal clades,

with various ways of locomotion, moving through different media (Alerstam et al. 2003, Alerstam 2006). Migration occurs in mammals (e.g. whales, large herbivores, bats), birds, reptiles (e.g. sea turtles), fish (e.g. salmon, cods), insects (e.g. Red Admiral butterfly) and marine invertebrates (Alerstam et al. 2003). It has also been suggested that migratory behaviour has a very deep evolutionary history: in volant species the first large scale movement potentially dating back as long as flight or seasonality itself (Rappole 2013). Migratory behaviour appears to be relatively flexible both in time, space and phylogeny. For instance, the lack of phylogenetic constraint on the evolution of migratory behaviour is illustrated by divergent migratory behaviours of closely related species, or of different populations of a single animal species (Alerstam 2006, Cristol et al. 2013). Present day migratory behaviour also shows considerable flexibility, by representing a dynamic, ever-changing behaviour; we occasionally witness sudden switches or gradual changes from migratory to resident or from resident to migratory strategies in certain animal species (Fiedler 2003). A well-documented case of switch in migratory behaviour occurred in blackcaps (*Sylvia atricapilla*) during the second half of the 20th century. Central European populations traditionally overwintered in western Mediterranean areas, but the number of individuals overwintering in Britain dramatically increased after the 1950s from a couple birds to several thousand individuals within only 30 years (Berthold et al. 1992).

Not only migratory strategy varies widely across species, but also the length and type of journey migrant species undertake (Newton 2008). Some birds only perform relatively short intra-regional or altitudinal movements, while others migrate across continents and travel over 10,000 kilometers (Rappole 2013). Migratory behaviour also varies in terms of continuity. Some species travel by several, relatively short jumps, with frequent stop-overs used for resting and refuelling, while others cover their entire journey with a single non-stop flight (Kirby et al. 2008, Gill et al. 2009, Hedenström 2010). The nature of migratory strategies adapted by diverse species largely define the energetic cost, the level of physiological stress as well as the

risk they face during their travel between their breeding and wintering grounds. Similarly, the physiological status of the migrants might determine their movement decisions.

Among migratory animals, birds are represented by a disproportionately high number of species, given that an estimated 19%, circa 1,855 of the world's 9,856 bird species is considered migratory (Kirby et al. 2008). Birds on the move are highly conspicuous, the migratory routes of which enmesh almost the entire globe (Newton 2008, Rappole 2013). This is partly the reason why our most detailed understanding of migratory behaviour comes from this group of animals. Nonetheless, other factors also elicited the study and understanding of avian migration. Primary among these is that bird watching is extremely popular, especially in the United States and Europe, and therefore not just scientists, but also members of the public contribute to the field by registering the movements of birds across both space and time. Second, bird ringing has a well developed methodology and has been ongoing ever since the end of the 19th century with ever increasing intensity. Currently, almost four million birds are ringed annually in Europe, with around 900,000 individuals marked in the U.K. alone (Baillie et al. 2007, 2009). Moreover, the study of avian migration was quickly advancing once migratory restlessness, its timing and orientation was described as a useful tool in investigating bird movements (Mewaldt & Rose 1960). Studying migratory restlessness and homing behaviour in domesticated birds (e.g. pigeons) has long traditions, well developed methodologies that nowadays enables scientists, for instance, to alter photoperiod, magnetic fields or even celestial cues in order to study orientation or migratory decisions. Satellite tracking recently added a new dimension to the study of animal movements. Using microelectronic tracking devices or transmitters allowed scientist to track individual movements with very high resolution over thousands of kilometers (Rappole 2013, Baillie et al. 2009). The latter technique again works especially well for terrestrial species that do not submerge waters and do not usually enter shelters that prevent satellite communication during their migration, such as birds. Additionally, in recent years molecular or chemical methods are being

used increasingly to characterise migration and movement of animals from insects to mammals. Such methods include analysis of stable isotopes (Hobson 1999) or DNA (Wink 2006) and which help to answer questions regarding the origin of birds, the location of their moult, characteristics of their nutritional resources and therefore wintering and breeding areas or stopover site locations. Moreover, fine scale movements, such as dispersal, immigration and emigration rates, population genetics are often much easier to describe using molecular analysis than traditional capture-recapture methods (Wink 2006). Advancements in related techniques, as well as the increasing availability of detailed spatial movements for multiple taxa, as well as the development of powerful analytical approaches, statistical methodologies especially during recent decades has led to more detailed understanding of migratory behaviour than ever before, but numerous questions remain open that need further scientific attention.

Besides satisfying our general biological curiosity, studies of migratory behaviour can help us to resolve a range of important practical issues concerning biodiversity conservation, global environmental change or public health. Birds on the move face a number of hazardous situations, involving habitat and stop-over site destruction, pollution, hunting, trapping or interference with air traffic (Fernández & Lankn 2008, Higuchi 2012). These dangers faced by migratory birds lead to their large-scale population declines and has the potential to result in major biodiversity loss (Both et al. 2009, Laaksonen & Lehikoinen 2013). Therefore, improved conservation efforts are needed to ensure the future and safety of migratory species, that can only be achieved if migratory behaviour is well understood and species-specific information is available on the spatial and temporal scale of their movements. Moreover, bird migration has considerable public health implications, due to the ability of migratory birds to spread zoonotic diseases (Baillie et al. 2009, Reed et al. 2003). Birds are homeothermic vertebrates that can serve either as reservoirs or as long-range dispersers of pathogenic microorganism, viruses, parasites that have the potential to infect humans, domestic animals or local wildlife. These include the West

Nile virus, *Borrelia burgdorferi*, influenza A virus, enteric bacterial pathogens, and drug-resistant bacteria (Reed et al. 2013). A better understanding of such diseases with migratory behaviour, might enable us to predict, prevent, control or treat zoonotic diseases with a higher efficiency.

Why do birds migrate?

The apparent lack of phylogenetic constraints on migratory behaviour, the disparate migratory strategies of different populations in a given species, the highly diverse movement patterns of closely related taxa or the witnessed transitions of migratory strategy suggest that migratory behaviour has little genetic constraints. Therefore, it was suggested by previous studies that the answer to the question of why birds migrate rather lies in proximal factors, and need rather be sought in the ambient (Berthold et al. 2013). Nonetheless, it is known that a significant proportion of the phenotypic variance in migratory traits is of genetic origin (Liedvogel et al. 2011). It is indeed widely accepted that migratory behaviour is the result of natural selection, migratory behaviour or even the migratory route undertaken is heritable, variable and has fitness consequences (Liedvogel et al. 2011). Migratory behaviour therefore appears to be a rather fast evolving life-history strategy, where new strategies can quickly outcompete the previous ones, for instance by advantages in breeding condition, earlier arrival, territory occupation or by a better match with peak food availability. The most important components determining the evolution of migratory behaviour include mortality and fecundity. Both of these life-time fitness components are largely affected by migratory behaviour, as food supplies, energetic costs of travel or local self-sustenance, dangers faced and fecundity all vary between wintering and breeding ground in the case of migrants (Alexander 1998).

Scientists were trying to answer why do animals perform migratory behaviour for millennia. The earliest proposed explanation suggested the role of

temperature. Birds migrating south to avoid cold winters and migrating north to avoid coming heat was proposed by Aristotle as early as 350 BCE (Somveille et al. 2015). It took more than two millenia until this theory was started to be rejected, mostly based on quantitative analyses showing that the number of migrants breeding in certain areas are not explained by temperature alone on these grounds (MacArthur 1959). As an alternative hypothesis, seasonality in food supplies was proposed as the most reasonable explanation for the evolution of migratory behaviour (MacArthur 1959). Therefore, it is not the absolute amount of resources available locally during the breeding season, but rather the intra-annual variability of resources and seasonality in productivity that emerges as the most important determinant of local species richness during breeding (Dalby et al. 2014). It was therefore deduced that the main factor determining the diversity and abundance of migrants breeding locally is explained by the surplus of resources not exploited by local, year-round resident species and is therefore available for incoming migrants (Somveille et al. 2015). An important component of this seasonality is rooted in the low temperature extremity on the breeding ground during the non-breeding season. Increasing winter harshness increases the energetic expenditure of residents, due to increased basal metabolism as well as energetic requirements for feeding, while resources become harder to access and more scarce, forcing most species to move on (Carnicer & Díaz-Delgado 2008). Therefore, temperature is actually important, but it's effect appears to be rather indirect, being mediated by resource availability (Somveille et al. 2015). A third major driving mechanism shaping migratory behaviour among species and individuals is the energetic constraint of migration. It was therefore suggested, that birds choose the closest suitable wintering region in order to decrease the energetic costs of travel (Somveille et al. 2015). Long-distance migration is one of the most extreme physiological stresses that birds experience during their lifetimes (Weber 2009, Hedenström 2010). Moreover, it was suggested that the probability of survival decreases with the length of migratory journey to be covered (Somveille et al. 2015).

Costs of migratory behavior and ways to minimise them

Generally speaking, no specific adaptation appears to exist in birds that has evolved solely for, or would serve as the single function of making migratory behaviour possible. Orientation skills, flight efficiency or metabolic requirements of the migratory flight are often all met in resident bird species just as well as in migrants (Berthold et al. 2013). Nonetheless, birds possess a whole range of preadaptations that makes them the most frequently encountered migratory animals and enables them to perform some of the most extreme migratory journeys ever recorded in the animal kingdom (Newton 2008). The most important amongst these adaptations is their ability to fly efficiently over long distances, a character that paves the road towards the evolution of large-distance migration. First, flight is the fastest way of locomotion, faster than walking, running or swimming, but at the same time is relatively economic in terms of the amount of energy consumed per unit distance travelled. For instance, large bodied soaring and gliding birds are capable to travel tens to hundreds of kilometers without a single wingbeat and consume little more energy than when sitting still (Newton 2008). Second, flight enables animals to cross geographic regions that would often constitute ecological barriers to migration, such as seas, mountain ridges or deserts. Third, flight is potentially the safest way of locomotion in respect to predation. In sum, flying offers the ability for a fast and energetically economic long-distance displacement, which elicits the exploitation of seasonal resources but at the same time leaves enough time and energy for reproduction and self-maintenance, enabling long-distance avian migration to be a successful life-history strategy (Weber 2009).

Anatomical adaptations to flight are numerous, the most striking of which include feathers, with flight feathers playing a particularly significant role in this respect by providing air-foils and enabling birds to take on wing. Active flight is aided by the enlargement of specific bundles of muscle fibres (e.g. pectoral, supracoracoidal

muscles) and of the bone surfaces where these muscle bundles are anchored. These enlarged flight muscles provide force and resistance during sustained migratory flight. Additional morphological characters of volant birds improve flight performance, such as light skeleton and hollow bone structures, the reduction in the number of bones by fusion and the lack of jaw and teeth that all contribute to the observed decrease in skeletal mass compared to similar sized mammals, decrease in wing loading and therefore in the power requirements of flight (Pennycuick 1975).

Besides anatomical adaptation, physiological characteristic observed in birds have similarly high importance in facilitating migratory behaviour. Primary amongst these is the unique metabolism that birds use in order to sustain high intensity, endurance locomotion. For instance, birds possess a highly efficient respiratory system that is fundamentally different from the mammalian respiratory setup which allow far more efficient gas exchange. This is especially important, due to the elevated metabolic needs of birds. Gas exchange efficiency is particularly important in birds flying at higher altitudes, where the partial pressure of oxygen compared to low altitudes and gas exchange needs higher efficiency to maintain required blood oxygen levels (Klaassen 1996). Moreover, heart size was also mentioned to reflect the need for cardiac output and therefore physical endurance capacity, such as required during migration (McWilliams et al. 2004).

A range of morphological adaptations that had been suggested by aerodynamic theory or empirical observations to reflect adaptations to migration are numerous. Nonetheless, these predictions have rarely been tested, especially in a comparative framework, where phylogenetic non-independence of species is taken into account. Moreover, studies often only focus on a limited number of organs or anatomical features, although these adaptations might be strongly correlated and non-independent, therefore their effect is ideally tested in a single research framework. Therefore, we performed a study investigating how wing morphology, and the size of exercise and nutritional organs covaries with migration distance across species (**Paper I**).

Another astonishing aspect of migrating birds is their dramatic adaptive organ size adjustment in line with their seasonal function requirements. Thus, increase in body mass in flying birds has a wide range of disadvantages ranging from elevated metabolic expenditure to increased risk of injury or predation, impaired foraging and take-off ability, as well as impaired flight performance (Witter & Cuthill 1993, Biebach 1996). Therefore, birds keep their body masses at minimum at any time by means of decreasing the size of their organs that are temporarily unused. For instance, testes size can be regressed 100 to 200-fold in starlings (*Sturnus vulgaris*), compared to its fully functional size at the peak of the breeding season (Dawson et al. 2002). Although similar physiological changes also occur in the gonads of seasonally breeding terrestrial mammals, their magnitude of change is far smaller (e.g. 3-fold decrease in soy sheep, Lincoln & Short 2013). Moreover, birds not only adjust the size of their gonads, but most of their organs also exhibit high plasticity in size, their changes occur at fast rates, which are reversible and temporary (Piersma & Lindström 1997). The reason behind the change is that the energetic needs of some vital organs (e.g. heart, kidneys, intestines) make up a major part of the energy consumption and basal metabolic rate of an individual (Piersma et al. 1998, 1999). Paying such costs during the already stressful migratory flight might drain resources prematurely and could easily lead to fatalities. Moreover, the weight of these organs represents extra load to be carried with no particular benefit, being non-functional at times. The extra load of non-functional organs can significantly increase wing loading, therefore the power requirements and risks faced during migration are also elevated (Witter & Cuthill 1993, Biebach 1996). Therefore, birds aim to adopt organ sizes to actual metabolic, reproductive or exercise needs in order to minimise current metabolic expenditure, especially during the extreme physiological stress of the migratory journey. Such plastic changes occur in multiple phases. For instance, during the early phases of pre-migratory preparation and fuelling process heart, liver and stomach mass increases in order to increase digestion efficiency of the excess food intake and to enhance energy storage (Piersma & Lindström 1997). During the middle period of

the fuelling process leg muscles, intestines, liver and kidneys increase, while stomach mass starts to regress. Later, during the last part of the pre-migratory fuelling process stomach mass regresses up to 50% (e.g. in red knots, *Calidris canutus*), in order to reduce body mass for the migratory flight (Piersma & Lindström 1997). Red knots have a particularly large stomach, taking up around 10% of lean body mass when fully developed. A 50% reduction in the stomach mass of these birds results in a 5% reduction of lean body mass, which leads to significant energy savings (Piersma & Lindström 1997). Moreover, prior departure leg muscles, intestine, stomach and liver masses all decrease in size, reflecting decreased needs for nutritional organs. Parallely, exercise organs, such as the pectoral muscle and heart size increase in preparation to the high intensity endurance flight (Piersma & Lindström 1997).

Strategic size adjustment of organs can reduce the costs of self maintenance during migration. Nonetheless, some organs are not flexible in size, therefore their costs have to be mitigated by other means, also resulting in loss of functionality. Such organ is the central nervous system, especially the brains of birds (Battley et al. 2000). The brain is the second most energetically expensive organ of the body (after the heart), consuming up to ten times more energy per unit mass than skeletal muscle (Isler & van Schaik 2006, 2009). Benefits of a large brain relative to the body has various advantages, including increased survival, adaptability to novel environments, innovation propensity, variability of habitats occupied, invasiveness and sociability (Lefebvre et al. 2004, Lefebvre & Sol 2008, Sol 2009, Sol et al. 2007, 2010, Lefebvre 2013). Despite these advantages, brain size evolution is limited by its large energetic costs. Given this burden, it is not surprising that migratory birds, experiencing high levels of physiological stress during migration, often have smaller relative brain sizes than residents or even short-distance migratory species. This association has repeatedly been demonstrated at the species level in birds (e.g. Winkler et al. 2004, Sol et al. 2005, Vincze et al. 2015), and bats (McGuire & Ratcliffe 2011), as well as at the subspecies level in birds (Cristol et al. 2003, Pravosudov et al. 2007, Fuchs et al. 2015). The correlation is very robust and, in all cases, brain weight relative to body

mass decreases with increasing migration distance (Cristol et al. 2003, Winkler et al. 2004, Sol et al. 2005, Pravosudov et al. 2007, McGuire & Ratcliffe 2011, Vincze et al. 2015).

Two mutually non-exclusive hypotheses had been proposed to explain this association. These by definition explain variation on two different ends of the migratory distance spectrum (i.e. residents vs. long-distance migrants). The energetic trade-off hypothesis builds upon the metabolic costs of migration, and that of developing and sustaining neural tissues, suggesting energetic conflict between these two demands (Winkler et al. 2004, McGuire & Ratcliffe 2011). Migration is one of the most energetically challenging periods in a bird's life, which is often on the edge of their physiological endurance and it necessitates a range of adaptations to make the journey possible (Hedenström 2010). Given the energetic cost of flight, long-distance migration may compromise a bird's ability to support the high metabolic cost of a large brain. Thus, the energetic trade-off hypothesis predicts directional selection that favours smaller relative brain size with increasing migration distance. The behavioural flexibility hypothesis on the other hand assumes a positive directional selection on relative brain size in resident birds (Winkler et al. 2004). Year-round residents often experience strong spatial and temporal fluctuations in their environments, and therefore tend to rely more heavily on novel food sources, exploited through innovations and learning (Sol et al. 2005, Aplin et al. 2013). Indeed, innovation propensity and associated relative brain size (Timmermans et al. 2000, Reader and Laland 2002), are both highest in resident species and lowest in long distance migrants (Sol et al. 2005).

In order to test the validity and setting of the above two hypotheses, I explored the nature of the negative correlation between relative brain size and migratory behaviour using data on 1,466 globally distributed bird species. I tested the validity of the energetic trade-off and behavioural flexibility hypotheses separately, using only long-distance migrants and resident bird species respectively (**Paper II**).

The association between migration distance and relative brain size in birds

appears to be general evolutionary phenomena, occurring both in birds and mammals (e.g. bats) from various taxonomic groups. Smaller relative brain size in migratory species might be the result of a similar magnitude shrinkage in all brain regions or of disproportional decrease in the size of particular brain regions. Indeed, brains are composed of numerous functionally distinct regions and therefore selection might cause selective enlargement or reduction of such neural substrates linked to particular behaviours or physiologies (Barton & Harvey 2000). The function of all three main brain divisions of the avian brain (i.e., telencephalon, cerebellum, optic lobe) the size of which might be related to migratory behaviour. Diverse information important for migration are projected and processed in nuclei of the telencephalon, such as spatial cues (hippocampus), magnetoreception and night vision (cluster N), audition (auditory cortex), olfaction (olfactory bulb), visual cues (visual Wulst, entopallium) and putative non-compass magnetic map information (trigeminal nerve recipient hindbrain nuclei, Fuchs et al. 2014, Healy et al. 1991, Sherry 2006, Heyers et al. 2007, Zapka et al. 2009). Nonetheless, regions responsible for these functions represent only a fraction of the entire telencephalon and its size might vary in response to a range of other factors. The optic lobe is another particularly conspicuous component of avian brain. It is part of the mid-brain and is well developed in birds (Butler & Hodos 2005, Wylie et al. 2009). The highly elaborated, multi-layer supraventricular part of the optic lobe is called the optic tectum. This surface area is mainly retinorecipient, receiving up to 90% of all visual information which is part of the primary visual pathway in birds (Wylie et al. 2015). Nonetheless, it also receives other sensory information, which together form a topographic (retinotopic) map in this brain region (Wylie et al. 2015). Deeper layers of the optic tectum contain motoric neurons, that aid quick eye and head movements, especially toward salient environmental stimuli, help visual discrimination, spatial positioning of stimuli, and motion processing without the need of higher cognitive processing (Butler & Hodos 2005, Wylie et al. 2009, Shimizu & Bowers 1999). The optic lobe might therefore play an important role in navigation, especially during migration, although such an

association lacks evidence. The third main brain region, the cerebellum is well developed in birds and serves as motoric control to skeletal muscles. A well-developed cerebellum would imply fine motor dexterity, higher motion precision, better coordination and timing during flight (Kaas 2009). Here we performed a phylogenetic comparative study in order to test how brain regions listed above covary with migration distance and to explore whether the brain size reduction in migrant compared to resident birds implies a uniform or non-uniform size reduction of these brain components (**Paper III**).

Seasonal organ size adjustment and inherently smaller brain size are just some of birds' major strategies to minimise their load to be carried during flight. An additional adaptation regards their metabolic pathway itself. Some birds during endurance flights, such as migratory flights, function at a very high metabolic rate, using the highest ever recorded mass-specific energy requirements among vertebrates, about twice as high as the maximum level ever recorded for exercising mammals (Jenni-Eierman & Jenni 1992, Jenni-Eiermann et al. 2002, Weber 2009). To cover such high metabolic expenditures, migratory birds use almost exclusively fatty acids, derived straight from adipose tissue to the working muscles by the circulatory system (Guglielmo et al. 1998). This character is a significant innovation compared to mammals, whose muscle work is mainly fuelled by blood glucose, muscle glycogen and intramuscular fatty acid stores during sustained and intense exercise (McWilliams et al. 2004).

Exercising mammals fuel their muscles with a combination of carbohydrates and fatty acids, with only a minor protein contribution (McWilliams et al. 2004). The proportion of the main two fuel types depends on the intensity of the exercise, or rather on the actual percentage of maximal oxygen consumption (% VO_{2max}). During low intensity exercise, such as walking (i.e. around <40% VO_{2max}) fatty acid metabolism can cover the entire energetic expenditure of the muscles. Nonetheless, as the intensity of the exercise increases the amount of energy delivered from fat metabolism decreases to only about 20%, while 80% is covered from carbohydrate

oxidation, derived mainly from the intramuscular glycogen stores. However, these stores are rather limited, and once these are depleted, exercising mammals are unable to sustain high intensity muscle work and fall back to the maximum level of exercise intensity that can be fuelled solely by fatty acid oxidation (McWilliams et al. 2004). This phenomenon is commonly known as “hitting the wall”, and is best studied in marathon runners (Rapoport 2010). Moreover, a more detailed examination of mammalian fatty acid metabolism at high intensity exercise revealed that only 25 – 50% of fat metabolised originates from exogenous adipose tissue delivered by the circulatory system and mostly originates from intra-myocyte triglyceride stores (Guglielmo et al. 2002). Birds on the other hand exercise at a very high intensity, achieving approximately double of the mammalian VO_2 max, which exercise is sustained for over days during migration, an activity unimaginable for mammals (Guglielmo et al. 2002). To achieve this, birds are able to fuel high intensity endurance flights in a way that is considered unique to flying vertebrates: muscle work in birds is predominantly fuelled by fatty acids delivered to the working muscles from extra-muscular adipose tissues (McWilliams et al. 2004). To achieve this, fatty acids in birds are being mobilised, transported and oxidised at rates much higher than in mammals. However, this aspect of the metabolism in migratory birds is poorly understood to date, mostly due to sampling difficulties of birds on the move (Jenni-Eiermann et al. 2002). Although little data is available on migratory bats, their metabolism appears to represent one of the finest examples of convergent evolution as means of adaptation to endurance flights (Guglielmo 2010). Lipid metabolism in bats is upregulated during the migratory period, similarly to migratory birds (McGuire et al. 2013), making metabolism of bats far more similar to birds than to cursorial mammals.

The benefit of using lipids as the core fuel of sustained flight is multifaceted. Carbohydrates and proteins contain 70-80% water, while fatty acids are highly hydrophobic, they are stored in almost anhydrous state, in the form triacylglycerol containing only 5% water, none of which is in free form, therefore they are lighter in

weight (McWilliams et al. 2004, Weber 2009, Jenni & Jenni-Eierman 1998). Moreover, fatty acids are chemically more reduced than proteins or carbohydrates, therefore their oxidation yields approximately twice as much energy per unit dry mass than the latter two energy sources (McWilliams et al. 2004). Owing to these characters, fatty acids represent the lightest energy storage form, resulting in a lower fuel load to be carried if the required amount of energy is stored in adipose tissue rather than alternative oxidative fuel types during migration (Weber 2009). Another previously suggested advantage of the chemical reduced state of fatty acids is that their oxidation yields more metabolic water than the oxidation of carbohydrates or proteins of similar weight. Indeed, oxidation of metabolic fuels yields 1.07 g of water per 1g of fat, and only 0.56 g or 0.40 g of water per 1g of glycogen and protein, respectively (Edney 1977). This aspect was thought to be significant, as it is not only energy that is limiting long-distance migratory flights, but also the availability of water, and therefore the risk of dehydration upon covering geographical barriers or inhospitable areas, such as seas or deserts (Klaassen 1996). Water loss in birds results from excretion and evaporative cooling (i.e. respiratory and cutaneous evaporation), while water imbalance occurs when the amount of water intake (both exogenous or metabolic origin) and loss are unbalanced (Landys et al. 2000, Klaassen 1996, Jenni & Jenni-Eierman 1998). Whether lipid metabolism is beneficial in terms of water balance was later questioned, as it was demonstrated that fat requires more oxygen to be metabolised, therefore water loss in this case is also higher due to higher respiratory flow (Klaassen 1996). As a result net water gain from lipid metabolism is approximately six times lower than that of protein metabolism per unit amount of energy obtained and therefore it is unlikely that fatty acids are the main source of water during migration. It is more likely that water imbalance is compensated by the protein catabolism observed during migration (Klaassen 1996, McWilliams 2004). Nonetheless, birds are often able to alter the time, altitude or the route of their migration in order to decrease water loss (Schmaljohann et al. 2009), therefore what role fat metabolism plays in keeping water balance needs further scientific attention.

It is a common strategy of most if not all bird species to store energy in times when resources are patchy, seasonal, unpredictable or when a significant energy expenditure is foreseeable (Rappole 2013). Such stores are usually composed of fat and is usually stored in large quantities preceding fledging, dispersal, migration, prior to wintering in resident species or prior to egg-laying in females (Biebach 1996, Phillips & Hamer 1999, Cox et al. 2011, Durant et al. 2010, Rappole 2013). In the case of migratory fuelling, energy is stored in sub-cutaneous fat and it is accumulated during pre-migratory fattening. During this period birds consume food in excess and gain weight at a very fast rate, with maximum deposition rates of 10-15% of lean body mass per day (Lindstöm 1991). Fuel stores are especially important and they reach their maximum levels before long-term non-stop flight, usually over inhospitable areas, such as seas and deserts, when birds rely entirely on their stored reserves (Bairlein 2003). For example, migratory birds commonly store large reserves of fuel prior to crossing the Sahara Desert or the Gulf of Mexico (e.g. Odum & Connell 1956, Berthold 2001). These migratory fat stores can reach 100% of lean body mass, leading to a doubled body mass only within a short period of one to three weeks during pre-migratory fattening (Odum & Connell 1956, Hedenström & Alerstam 1992, Biebach 1996, Newton 2008).

Accumulated fuel mostly comprises lipids - the primary fuel of migratory birds. Besides being energy-dense, fats aid thermoregulation, social signalling, impart mechanical protection, as well as buoyancy and are vital during migration (Mortensen & Blix 1986, Witter & Cuthill 1993, Lind et al. 1999). Nonetheless, fat reserves also confer a wide range of disadvantages. These include the risky and energetically demanding process of fuel accumulation, costs emerging due to the increased body mass, and increased locomotor energy requirements (reviewed in Witter & Cuthill 1993). The aerodynamic implications of fat load accumulated are also multifaceted: the fuel deposited subcutaneously increases the projected frontal area, which increases body drag, decreases lift-to-drag ratio, and increases power required for flight (Pennycuick 1975, Hedenström & Alerstam 1992, 1997, Hedenström 2010).

Secondly, the accumulation of fuel increases wing loading, which increases the cost of lift (Pennyquick 2008, Hedenström 2010).

The subcutaneously accumulated fuel preceding migratory flight is very conspicuous in birds, and their amounts vary widely among species and individuals. This variation was suggested to be related to the length of migratory journey the bird is preparing for, but this hypothesis has not been tested in a comparative framework. Moreover, after controlling for the length of migratory journey, a considerable variation remains in reserve sizes across species. What behavioural or anatomical components explain this variation are little known. Here we performed a study on maximum fuel store sizes across birds and explored these questions (**Paper IV**).

Objectives and major results

Here I outline the specific objectives, questions and predictions of the studies included in this thesis. I briefly summarise the methodology used, including data collection, statistical procedure and detail the most important results achieved. Note that these studies are described in details in Chapter II.

Paper I *Morphological adaptations to migration*

(i) Objectives

Migratory birds often cover thousands of kilometers on their migratory journey, often by means of long spell non-stop flights. Such sustained high-intensity exercise necessitates multifaceted adaptations to be realised. Indeed, aerodynamic theory predicts a range of morphological and anatomical adaptations that serve the role to minimise the costs of migration. Most of these adaptations, however, was never explored in a phylogenetic comparative setup that would account for the correlated nature of multiple exercise or metabolic organs. Here we assembled a unique dataset of 149 European bird species, comprising their migration distance and a range of morphological characters. These included wing morphology (wingspan, wing loading, wing area, wing aspect ratio), exercise organ sizes (pectoral muscle, supracoracoidal muscle, heart) and digestive organ sizes (liver, gizzard). We expected that wing aspect ratio and wingspan will increase, while wing loading will decrease with increasing migration distance. Moreover, we expected an increase in the size of exercise organs and a decrease in the size of nutritional organs with increasing migration distance. We used multivariate phylogenetic generalised least squares (PGLS) models, incorporating a variance-covariance matrix the structure of which is based on the evolutionary history of the species represented in the dataset. We used

migration distance as a dependent variable and organ sizes, wing morphology as covariates.

(ii) Results

Our results show that (1) aspect ratio increased, while relative heart weight and wing loading decreased with increasing migration distance. (2) These results were robust to whether the analyses were based on the entire species pool or limited to passerines or to a species pool excluding residents. The association between migration distance and exercise (except of heart size) or nutritional organ sizes was less clear. Nonetheless, we warn against clear conclusions on the lack of association between organ sizes and migration distance, due to the methodological difficulty to measure these organs given their high flexibility in size over the year or according to their current workload. (3) Taken together, these results point toward the importance of morphological adaptations that reduce the energetic expenditure during migratory flight and increase flight range in migratory birds.

Paper II

Brain size evolution and migratory behaviour in birds

(i) Objectives

Brain size relative to body size has repeatedly been demonstrated to be smaller in migratory than in resident species of homeothermic vertebrates. The association has been described across species in birds and bats, as well as within species between migratory and sedentary subspecies. Therefore, the negative correlation between overall migration distance and relative brain size appears to be adaptive, and is best described as a negative trade-off between migration and brain size. Two, mutually non-exclusive hypotheses had been proposed to explain this negative trade-off. First, the *energetic trade-off hypothesis* is based on the high energetic demands of both brains and migratory flights. Indeed, the brain is one of the most energetically demanding organ of the body, while migration is one of the most

demanding periods of a bird's life. This hypothesis, thus argues that the nature of the negative association between relative brain size is purely energetic. Second, the *behavioural flexibility hypothesis* argues that resident species will be selected to have larger relative brain size in order to cope with more seasonal environments and fluctuating resource availability. Indeed, relative brain size is a strong predictor of innovativity, social learning, information processing and storage among free ranging birds. Using literature, here I collected data on brain mass, body mass, migration distance, wintering minimum temperatures, seasonality and wintering latitudes of 1,466 bird species. I constructed PGLS models incorporating phylogenetic relatedness across species. I built separate models for resident and migratory species, including environmental variables or migration distance respectively to test the two hypotheses mentioned above.

(ii) Results

My results indicate that (1) the previously established negative correlation between brain size and migration distance is strong and can be generalised to a species pool of very wide taxonomic coverage. (2) Using only long-distance migratory species, I demonstrate that the energetic trade-off hypothesis is supported and appears to be a major contributor to brain size evolution among migratory species. This is also supported by analyses restricted to long-distance migratory species with tropical wintering areas. (3) Using the only resident species I demonstrate that winter minimum temperature negatively correlates with relative brain size, indicating a positive selection for larger relative brain size in harsh environments. In comparison, wintering latitude or seasonality appeared to be weaker predictors of relative brain size. Together, these results illustrate that both of the above mentioned hypotheses are supported, and these two selective forces are likely to act on two different ends of the migration distance spectrum.

Paper III *Brain regions and their association with migratory behaviour in birds*

(i) Objectives

Despite our detailed understanding on the function of the three main brain regions of birds (i.e. telencephalon, optic lobe and cerebellum) and their suggested roles in migratory flight, our knowledge on the mosaic evolution of avian brains with migratory distance is limited. It is known that relative brain size of migratory species is smaller than in residents, nonetheless whether reduction in brain size with migration distance can be generalized across the different brain regions is less known. To investigate this question, here we collected data from literature on brain region sizes of 152 bird species, belonging to 61 avian families from six continents. Data covered optic lobe, telencephalon, cerebellum and whole brain size, body mass and migration distance. We built PGLS models and controlled for common descent. We used migration distance as a dependent variable, brain region sizes and body mass as covariates. Using the results, we aimed to infer the relative importance of the three main brain regions for migratory species in contrast to residents.

(ii) Results

Our results indicate that (1) total brain size decreases with migration distance in our sample of species and this reduction is accounted for by reduction in telencephalon size. (2) On the contrary, the size of the optic lobe increased with increasing migration distance, and (3) the size of cerebellum does not change with the length of the migratory journey. These results together indicate that energetic needs of neural tissues can be largely subsidised by reduction in the telencephalon size. On the other hand, the optic lobe appears to play a more significant role in migrants than in residents, probably due to the higher significance of visual acuity, visual information processing or the retinotopic map information of various environmental stimuli in migratory birds.

(i) Objectives

Migratory birds are well known to accumulate large amounts of fat, protein and other nutrients prior their migratory departure. Such fuel is usually accumulated within a short (1-3 weeks) period prior migration and can exceed in weight the lean body mass, leading to a more than doubled body weight at the beginning of the migration. The amount of fuel varies largely between species of birds, and has strong fitness consequences. On the one hand stored reserves are often the sole source of energy in migratory birds, which have to cover the longest non-stop flight spell the species pursues. On the other hand, fuel load has negative effect on manoeuvrability, predator avoidance and flight dynamics, therefore might decrease survival if too much extra fat is stored. Fuel accumulation in migratory birds has previously been linked to the length of their migratory journey, nonetheless such association lacks evidence across species. To date our knowledge is limited on what factors, especially other than migration distance influence the cross-species variation in accumulated fuel loads. Within the framework of this phylogenetic comparative study, we collected maximum fuel load data from 139 European bird species and investigated how migration distance, wing morphology and flight style influenced maximum fuel load across birds. We quantified fuel load based on literature data and validated this measure using condition indexes calculated based on migrant birds captured in a ringing camp. We collected data on migration distance, wing morphology and flight style of these species. We built PGLS models where fuel load was used as a dependent variable, while migration distance, wing morphology and flight style were included as explanatory variables. We used information theory based model selection to evaluate the importance of these predictors.

(ii) Results

Our results indicate that (1) maximum fuel load strongly increases with the

length of migratory journey across species. Additionally, we showed (2) that species with high wing aspect ratio accumulate less fuel. (3) Wing loading and flight style have little predictive power for maximum fuel load across birds. Our results indicate that fuel load is determined by the length of migratory distance and the flight energy-efficiency of the species.

General discussion

In this section I discuss my main results, their connection to each other and I detail their implications and importance. More detailed discussions of the results will be found in the copy-edited publications listed in Chapter II.

Adaptations to migration

Migration is an energetically demanding activity for birds, long-distance non-stop flights often being assumed to be on the edge of physiological endurance of migrants (Weber 2009, Hedenström 2010). As a consequence, natural selection over physiology, morphology and behaviour of the migratory flight is expected to be strong, also illustrated by the high mortality rate often observed during migration, especially in the case of unexperienced birds or of individuals in poor body condition (Owen & Black 1989). Mortality is indeed higher during migration than during any other period of the year (Standberg et al. 2009, Guillemain et al. 2010). Our work concentrated on exploring some of the adaptations that might serve as the function of reducing migratory costs, enable long-spell flights, and how these adaptations relate to phylogeny and other aspects of life history across birds. Within the framework of the four studies presented here, we revealed key energetic, anatomical and behavioural adaptations to migratory behaviour in birds.

Importantly, we demonstrated that energetically expensive organs mostly decrease in size with increasing migration distance. Both heart, the most expensive organ of the body (**Paper I**) and brain size (**Paper II & III**), the second most expensive organ of the body significantly decrease in size with increasing migration

distance. In the case of the heart, this negative association is surprising, given that decrease in organ size also infers decrease in functionality (Piersma & Lindström 1997). Migrant birds are expected to have well-developed exercise organs, such as heart, lungs and flight muscles in order to sustain high-intensity endurance flights (Piersma et al. 2005). The association between migratory behaviour and heart size has previously been tested and the results showed no association between migratory behaviour and heart size (Winkler & Leisler 1992). Given that the association also holds for passerines alone, a group of birds exhibiting highly similar flight styles, it can be assumed that the nature of this association is probably an energetic trade-off and is not a by-product of variation in flight style or flight energetics across species. Nonetheless, heart size is also one of the organs that shows major size adjustment prior to migratory departure, therefore these results are to be treated with caution. Moreover, our results indicate that the two muscle bundles listed as the most important ones for flight, the pectoral and supracoracoidal shows no association with migration distance (**Paper I**). Similarly, organ sizes, such as the gizzard and the liver were unrelated to the length of migratory journey undertaken by different species. These results indicate that factors other than migration influence organ and muscles size evolution across species. For instance, flight style might be an important determinant of exercise and metabolic organs, given its importance in determining flight costs, energetics and speed. Nonetheless, further studies are needed to clarify the extent and dynamics of strategic size adjustment of all exercise and digestive organs listed here prior to migratory departure. Without such knowledge, no definitive conclusion can be drawn about organ size evolution as a function of migration distance.

A clearer pattern emerges in the case of brain size, a highly expensive organ that is inflexible in case of physiological stress and is fixed in size once the bird reaches adult age (Battley et al. 2000). My results demonstrate that relative brain size being smaller in migrants than in residents is general to birds, and is apparent even within specific bird orders exhibiting various migratory behaviours (e.g.

Charadriiformes and *Anseriformes*), passerines or in the case of the entire species pool of 1,466 species as well (**Paper II**). My results also illustrate that this relationship is most likely of multiple origins. First of all, the smaller brain size in migrants is partly the result of a trade-off between the energetic expenses of migratory flight and that of sustaining the high metabolic needs of neural tissue masses. This is well illustrated by the negative association between relative brain size and migration distance in long-distance migrants alone, i.e. in species with over 2,000 km migration distances. It was however suggested that the length of the migratory flight might covary with wintering condition, such as if longer distance migrants overwinter in warmer environments. Nonetheless, models restricted to long-distance migrants with wintering areas within the tropic belt, also showed strong negative association between migration distance and brain size. These results further support that this negative association originates from an energetic trade-off and is unlikely to be confounded by variation in wintering conditions across species. It is likely that a major directional selection occurs for smaller relative brain size in migratory birds, potentially by means of premature energy depletion during long flight spells, leading to fatalities. This selection however would by definition be most important in long-distance migratory species and it is expected to have the strongest effect on species at the long-distance end of the migratory spectrum (Hedenström 2010). At the other end of the migratory spectrum (i.e., in resident species) another factor contributes to the increase in relative brain size. My results illustrate that environmental conditions, especially low winter minimum temperatures, assumed to reflect environmental harshness, represent conditions under which large relative brain size are favoured by natural selection. Additionally, relative brain size significantly increased with non-breeding latitude and seasonality, although these effects are weaker than the association with non-breeding minimum temperature. Therefore, it is more likely that environmental severity reflected by low ambient temperatures, unavailability of usual food resources, high snow cover, and/or reduced day length (Roth & Pravosudov 2009, Estók et al. 2009), rather than the seasonal nature of the environment is the

strongest selective force on relative brain size evolution across resident birds. The importance of climate severity on brain size evolution has previously been demonstrated for instance in food-caching Black-capped Chickadees (*Poecile atricapillus*). This species from harsher wintering ranges have better spatial memory, as well as larger hippocampi and higher neuronal density in these brain regions responsible for this skill (Roth & Pravosudov 2009, Pravosudov & Clayton 2002, Roth et al. 2011). Moreover, environmental stochasticity has previously been demonstrated to be positively associated with relative brain size in neotropical parrots (Schuck-Paim 2008). Enhanced cognitive ability might enable species to be more successful exploiters of novel food sources, by higher propensity of innovation or learning which results in higher survival or fitness under fluctuating resource availability (Sol et al. 2005, Aplin et al. 2013).

Whether brain size reduction with increasing migration distance is in fact affecting cognitive skills and the evolution of higher mental abilities is yet to be proved, but a good number of results indicate this. For instance, our results point out that the change in the size of different brain regions with migration distance is non-uniform (**Paper III**, see also McGuire & Ratcliffe 2011, Fuchs et al. 2014). Reduction of whole brain size with increasing migration distance is mostly accounted for by reduction in the size of the telencephalon (**Paper III**), the centre of higher cognitive processes. In line with the latter results, Fuchs et al. (2015) found that migratory lark sparrows (*Chondestes grammacus*) showed a clear trend toward having smaller nidopalliums (a central neural substrate of higher cognitive processes in birds) than residents of the same species. Therefore, it is probable that the larger relative brain size of resident birds compared to migrants is indeed associated with their larger telencephalon and better cognitive abilities that could enhance their survival probability especially under harsh environmental conditions. It would be insightful then to consider how environmental harshness in various resident birds influences the evolution of different brain regions, on a cross-species scale. Such a follow-up study could provide more precise insights into whether increase in the size of telencephalon

(and regions thereof, e.g. hippocampus) is specifically selected in species wintering under harsher environmental conditions. Additionally, whether brain size enlargement preceded, or followed, the switch in migratory behaviour in avian evolution is yet to be determined. Pravosudov et al. (2007) for instance examined three subspecies of white-crowned sparrow (*Zonotrichia leucophrys*) and showed that it is more likely that brain size enlargement took place after the switch from migratory to sedentary behaviour. Nonetheless, further studies should examine the nature of these associations on a broader taxonomic scale.

Earlier studies (e.g. Piersma & Gill 1998, Piersma et al. 1999, Battley et al. 2000) and our works (**Paper I-IV**) all finely illustrate the importance of weight reduction during the migratory flight. Decrease in body mass is, however, achieved at high prices, such as reduction in brain size (**Paper II**), especially telencephalon size (**Paper III**) and therefore arguable loss of a highly important fitness component: cognitive potential. Moreover, unused organs and muscles are atrophied, that need to be rebuilt once the migratory journey is completed, while metabolism switches to mostly fatty-acid based, low-weight fuel types (Piersma & Gill 1998). Birds in this respect work highly similar to flight planning of aircrafts which reduce weight by reducing load to be carried, reducing energy requirements of flight and reducing fuel amounts. Our results indicate that fuel stores strongly increased with the geographic distance covered during migratory flight, and therefore the total expected energy expenditure of given species of birds (**Paper IV**). Moreover, fuel stores are also adjusted to fit wing morphology, specifically species with high wing aspect ratio accumulate less fuel prior their migratory departure, than species with low wing aspect ratio. The latter result arguably originates from the power required of flight, that strongly depends on flight style and wing morphology (Norberg 2012, Ricklefs 1996). High wing aspect ratio and low wing loading is often associated with more economic flight performance, such as soaring flight (Rayner 1988). This is well illustrated by the sustained flight of the extremely long and narrow-winged albatrosses, flying for hours with only minimal wing movements, consuming very

little energy (Newton 2008). Indeed, our results highlight that bird species with a more economic flight apparatus accumulate less fuel for their migration, irrespective of the length of journey they are covering. This result is the evidence of a fuelling strategy that ensures enough energy for the migration, but does not add a surplus of weight that would be carried without benefits. Indeed, induced power (i.e., the power required to maintain lift and overcome gravitation) and parasite power (i.e. the power required to overcome aerodynamic drag) both increase with increasing body mass (Norberg 2012). Therefore, the most economic fuelling strategy and arguably the one that provides the best survival prospects is to store enough fuel that safeguards from energy depletion, but is not an overload that will not be used (Milner-Gulland et al. 2011).

Flight style varies strikingly among different bird species with very different energetic requirements (Ricklefs 1996, Norberg 2012). Flapping flight is energetically the most demanding flight style, while soaring, gliding and alternating flapping and gliding flights, or behaviours, such as flying in formations help birds to obtain a lower power output (Ricklefs 1996). As a result, flight style differences across species is expected to lead to a highly divergent total energy requirement to cover a unit distance. Interestingly, our results show that flight style has no influence over the amount of fuel stored prior migratory departure in birds. The different energetic needs of various flight styles appear to be clear and well defined (e.g., Norberg 2012), nonetheless, the longest non-stop flight ever recorded for migrants comes from a continuous flapper species. The bar-tailed godwit (*Limosa lapponica baueri*) travels 11,000 kilometers in a single 9-days flight from Alaska to New-Zealand (Gill et al. 2009). Although godwits travel in flocks, which is often assumed to reduce the costs of flight, this effect is also minimised due to their flapping flight style (Hedenström 2010). How continuous flapping species reduce their travel costs is therefore a topic of further scientific interest. Additionally, it is worth mentioning, although the example of the bar-tailed godwits contradicts this, that flight style might be linked to other characteristics of migratory flight that mask the effect of flight style per se. For

instance, flight style might be related to the number of stopovers along the migratory route and to the total distance covered during a single flight. The shorter the distance that birds have to cover at once, the less fuel they need to carry. In case of continuous flapping, species are more likely to split their journey in small sections, this would result in a smaller maximum fuel store than what we expect from their flight style and total distance covered. Moreover, flight style is strongly related to wing morphology, as high aspect ratio and low wing loading is often associated with soaring flight (Ricklefs 1996), while these two wing morphology parameters might better capture fine-scale species differences in flight dynamics than flight style categories. Therefore, further studies are needed to explore the fine spatial and temporal pattern of migration across species, along with their wingbeat frequencies and other flights characteristics.

Concluding remarks

Migration is the most energetically demanding and hazardous activities of birds, that is often associated with extreme mortality rates and periods of strong natural selection (Weber 2009, Standberg et al. 2009, Guillemain et al. 2010, Hedenström 2010). Birds possess a whole range of extreme adaptations that safeguard them during these strenuous movements. Nonetheless, it is increasingly important for us to explore, study and understand all aspects of these adaptations and migratory behaviour in general, including proximate and ultimate drives, physiological background, energetics, spatial and temporal dynamics. Climate change, industrialisation and the increasing size of the human population are factors that together contribute to the shrinkage, destruction, fragmentation, exploitation or complete disappearance of some natural breeding, wintering or stopover habitats of many migratory species. In case any of these areas are affected, a decrease in the survival prospects of migrants is expected and often observed. Moreover, long-distance migratory species are less able to cope with climate change in terms of being less flexible to adjust their arrival and breeding time to the advanced breeding conditions (Both et al. 2009, Møller et al. 2008). Together these factors majorly contribute to the large-scale decline of migratory species, including mammals, birds, fish and invertebrates (Wilcove & Wikelski 2008). Even among migrants, long-distance travellers show the steepest recent population declines (Morrison et al. 2013). It is therefore of pressing need to understand diverse aspects of the migratory behaviour of animals, not just from scientific, but also from biodiversity conservation perspectives. A better understanding of this fascinating behaviour will hopefully aid us in controlling and saving species on the way of disappearance.

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CHAPTER II

In this chapter I present the published papers and the manuscripts that constitutes the basis of my thesis.

List of publications related to the dissertation

- I Vágási, C. I., Pap, P. L., Vincze, O., Osváth, G., Erritzøe, J., & Møller, A. P. (2016). Morphological Adaptations to Migration in Birds. *Evolutionary Biology*, 43(1), 48-59.
- II Vincze, O. (2016). Light enough to travel or wise enough to stay? Brain size evolution and migratory behavior in birds. *Evolution*, 70(9), 2123-2133.
- III Vincze, O., Vágási, C. I., Pap, P. L., Osváth, G., & Møller, A. P. (2015). Brain regions associated with visual cues are important for bird migration. *Biology letters*, 11(11), 20150678.
- IV Vincze, O., Vágási, C. I., Pap, P. L., Palmer, C., Dyke, G., & Møller, A. P. – Migration distance and wing morphology predict accumulated fuel load in birds. *Manuscript*.

Personal contribution to publications included in the thesis

	I	II	III	IV
Original idea		*		
Study design and data collection	*	*	*	*
Data analyses	*	*	*	*
Manuscript preparation	*	*	*	*



Registry number: DEENK/237/2017.PL
Subject: PhD Publikációs Lista

Candidate: Orsolya Vincze
Neptun ID: CQTLX5
Doctoral School: Pál Juhász-Nagy Doctoral School of Biology and Environmental Sciences
MTMT ID: 10043490

List of publications related to the dissertation

Foreign language scientific articles in international journals (3)

1. **Vincze, O.**: Light enough to travel or wise enough to stay?: Brain size evolution and migratory behavior in birds.
Evolution. 70 (9), 2123-2133, 2016. ISSN: 0014-3820.
DOI: <http://dx.doi.org/10.1111/evo.13012>
IF: 4.201
2. Vágási, C. I., Pap, P. L., **Vincze, O.**, Osváth, G., Erritzøe, J., Møller, A. P.: Morphological adaptations to migration in birds.
Evol Biol. 43 (1), 48-59, 2016. ISSN: 0071-3260.
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3. **Vincze, O.**, Vágási, C. I., Pap, P. L., Osváth, G., Møller, A. P.: Brain regions associated with visual cues are important for bird migration.
Biol. Lett. 11, 20150678-1 - 20150678-4, 2015. ISSN: 1744-9561.
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IF: 2.823





List of other publications

Hungarian scientific articles in Hungarian journals (1)

4. Vágási, C. I., **Vincze, O.**, Pap, P. L., Barta, Z.: Oxidatív stressz és öregedés madaraknál.
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Foreign language scientific articles in international journals (13)

5. Pap, P. L., **Vincze, O.**, Wekerle, B., Daubner, T., Vágási, C. I., Nudds, R. L., Dyke, G. J., Osváth, G.: A phylogenetic comparative analysis reveals correlations between body feather structure and habitat.
Funct. Ecol. 31 (6), 1241-1251, 2017. ISSN: 0269-8463.
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IF: 5.63 (2016)
6. **Vincze, O.**, Kosztolányi, A., Barta, Z., Küpper, C., AlRashidi, M., Amat, J. A., Argüelles Ticó, A., Burns, F., Cavitt, J., Conway, W. C., Cruz-López, M., Desucre-Medrano, A. E., Dos Remedios, N., Figuerola, J., Galindo-Espinosa, D., García-Peña, G. E., Gómez Del, A. S., Gratto-Trevor, C., Jönsson, P., Lloyd, P., Montalvo, T., Parra, J. E., Pruner, R., Que, P., Liu, Y., Saalfeld, S. T., Schulz, R., Serra, L., St Clair, J. J. H., Stenzel, L. E., Weston, M. A., Yasué, M., Zefania, S., Székely, T.: Parental cooperation in a changing climate: fluctuating environments predict shifts in care division.
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7. Vágási, C. I., **Vincze, O.**, Pătraș, L., Osváth, G., Marton, A., Bărbos, L., Sol, D., Pap, P. L.: Large-brained birds suffer less oxidative damage.
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IF: 2.792





8. Bulla, M., Valcu, M., Dokter, A. M., Dondua, A. G., Kosztolányi, A., Rutten, A. L., Helm, B., Sandercock, B. K., Casler, B., Ens, B. J., Spiegel, C. S., Hassell, C. J., Küpper, C., Minton, C., Burgas, D., Lank, D. B., Payer, D. C., Loktionov, E. Y., Nol, E., Kwon, E., Smith, F., Gates, H. R., Vitnerová, H., Prüter, H., Johnson, J. A., St Clair, J. J. H., Lamarre, J. F., Rausch, J., Reneerkens, J., Conklin, J. R., Burger, J., Liebezeit, J., Bêty, J., Coleman, J. T., Figuerola, J., Hooijmeijer, J. C. E. W., Alves, J. A., Smith, J. A. M., Weidinger, K., Koivula, K., Gosbell, K., Exo, K. M., Niles, L., Koloski, L., McKinnon, L., Praus, L., Klaassen, M., Giroux, M. A., Sládeček, M., Boldenow, M. L., Goldstein, M. I., Šálek, M., Senner, N., Rönkä, N., Lecomte, N., Gilg, O., **Vincze, O.**, Johnson, O. W., Smith, P. A., Woodard, P. F., Tomkovich, P. S., Battley, P. F., Bentzen, R., Lanctot, R. B., Porter, R., Saalfeld, S. T., Freeman, S., Brown, S. C., Yezerinac, S., Székely, T., Montalvo, T., Piersma, T., Loverti, V., Pakanen, V. M., Tijssen, W., Kempnaers, B.: Unexpected diversity in socially synchronized rhythms of shorebirds.
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9. Martin-Silverstone, E., **Vincze, O.**, McCann, R., Jonsson, C. H. W., Palmer, C., Kaiser, G., Dyke, G. J.: Exploring the relationship between skeletal mass and total body mass in birds.
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10. Pap, P. L., Osváth, G., Sándor, K., **Vincze, O.**, Bărbos, L., Marton, A., Nudds, R. L., Vágási, C. I.: Interspecific variation in the structural properties of flight feathers in birds indicates adaptation to flight requirements and habitat.
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11. Pap, P. L., Vágási, C. I., **Vincze, O.**, Osváth, G., Veres-Szászka, J., Czirják, G. Á.: Physiological pace of life: the link between constitutive immunity, developmental period, and metabolic rate in European birds.
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12. Pap, P. L., Osváth, G., Aparicio, J. M., Bărbos, L., Matyjasiak, P., Rubolini, D., Saino, N., Vágási, C. I., **Vincze, O.**, Møller, A. P.: Sexual dimorphism and population differences in structural properties of barn swallow (*Hirundo rustica*) wing and tail feathers.
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13. Lovas-Kiss, Á., Sonkoly, J., **Vincze, O.**, Green, A. J., Takács, A., Molnár, V. A.: Strong potential for endozoochory by waterfowl in a rare, ephemeral wetland plant species, *Astragalus contortuplicatus* (Fabaceae).
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Morphological Adaptations to Migration in Birds

Csongor I. Vágási^{1,2} · Péter L. Pap^{1,2} · Orsolya Vincze^{1,2} · Gergely Osváth^{2,3} · Johannes Erritzøe⁴ · Anders Pape Møller⁵

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Abstract Migratory flight performance has direct or carry-over effects on fitness. Therefore, selection is expected to act on minimizing the costs of migratory flight, which increases with the distance covered. Aerodynamic theory predicts how morphological adaptations improve flight performance. These predictions have rarely been tested in comparative analyses that account for scaling and phylogenetic effects. We amassed a unique dataset of 149 European bird species and 10 morphological traits. Mass-adjusted aspect ratio increased, while mass-adjusted heart weight and wing loading decreased with increasing migration distance. These results were robust to whether the analyses were based on the entire species pool or limited to passerines or migrants. Our findings indicate that selection due to migration acts on wing traits that reduce the energetic cost of transportation to increase the flight range. Consequently, the demands for high ‘exercise organ’

performance might be low, and hence such energetically expensive tissues are not associated (pectoral muscle) or are inversely associated (heart) with migration distance.

Keywords Aerodynamics · Functional morphology · ‘Migratory syndrome’ · Organ size · Phylogenetic comparative analysis · Wing morphology

Introduction

Flight capacity constitutes complex adaptations in homeothermic vertebrates: animals get airborne to migrate, forage, display, hunt or escape from predators. Extraordinary locomotion during migratory journeys is the one that fascinated scientists for a long time due to impressive distances covered (e.g. 64,000 km a year by sooty shearwaters *Puffinus griseus*; Shaffer et al. 2006) and high relevance for ecological, evolutionary, conservation and health considerations (e.g. Webster et al. 2002; Alerstam et al. 2003; Møller et al. 2008; Bowlin et al. 2010; Altizer et al. 2011; Bauer and Hoye 2014).

Birds exhibit a large number of morphological (e.g. body mass, bones, feathered wings, wing-powering muscles, streamlined shape) and physiological and visceral organic (e.g. respiration, cardiovascular system, metabolic rate) adaptations that are thought to enhance flight capacity (Piersma et al. 2005; Videler 2005). However, besides these ‘general flight adaptations’, Darwinian selection is expected to add further ‘adaptive layers’ that particularly optimize the net pay-off of migratory flight. This expectation is reasonable considering (1) the energetic and antioxidant expenses that migratory flight entails, mainly when covering long distances (Costantini et al. 2007; Klaassen 1996; Rayner 1990), (2) the substantial mortality

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✉ Csongor I. Vágási
csvagasi@gmail.com

¹ MTA-DE “Lendület” Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, Debrecen, Hungary

² Evolutionary Ecology Group, Hungarian Department of Biology and Ecology, Babeş-Bolyai University, Cluj Napoca, Romania

³ Museum of Zoology, Babeş-Bolyai University, Cluj Napoca, Romania

⁴ Taps Old Rectory, Christiansfeld, Denmark

⁵ Laboratoire d’Ecologie, Systématique et Evolution, CNRS UMR 8079, Université Paris-Sud, Orsay Cedex, France

rate during migration (Klaassen et al. 2014; Newton 2004), and (3) the huge variation in migration distance among species. Aerodynamic theory predicts how morphological adaptations might maximize performance through either energy-efficiency (i.e. transport cost minimization) or time-efficiency of migratory flight (Hedenström 2008; Norberg 1990, 1994). We apply this functional morphology paradigm for studying morphological adaptations to long-distance migratory flight in birds.

A flying bird should produce lift and thrust that exceed weight (i.e. pull of gravity) and drag, respectively, to maintain a forward momentum. Both lift and thrust are ensured by the feathered wings and their highly complex movement during the stroke cycle. Wing loading (i.e. weight/wing area) and aspect ratio (i.e. wingspan²/wing area) are two wing morphology variables considered to greatly influence flight capacity and economy. Aerodynamic theory predicts higher body mass and/or smaller wing area if time-efficiency (i.e. flight speed maximization) is selected for because flight velocity scales to wing loading to the 0.32 power (Alerstam et al. 2007), while higher aspect ratio and lower wing loading is expected to evolve if optimal flight economy (longest flight range for smallest flight power) is selected (Norberg 1990). Therefore, both temporal and energetic currencies of migratory flight depend on the size and shape of the wing. Long-distance migratory birds were reported to have higher aspect ratio, longer distal wing, pointed wingtips and lower wing-loading than short-distance migrants or residents (Calmaestra and Moreno 2001; Fiedler 2005; Kaboli et al. 2007; Leisler and Winkler 2003; Lockwood et al. 1998; Mönkkönen 1995; Voelker 2001; Winkler and Leisler 1992), which hints toward the importance of energy-efficient flight. However, these studies do not uniformly detect such correlations between migration distance and morphological traits and reached contrasting conclusions (reviewed by Mulvihill and Chandler 1990; Lockwood et al. 1998) about whether migration represents an important selective force that shapes avian external morphology (Leisler and Winkler 2003).

Visceral organs also play an important role in migratory flight because they contribute to body mass and metabolic rate (Piersma and Lindström 1997). Given that the performance of organs increases with their size (Piersma and Lindström 1997), migrants are expected to have well-developed ‘exercise organs’ such as flight muscles (pectoral and supracoracoid), heart and lung (Piersma et al. 2005). This should be favoured owing to (1) higher mechanical power production by larger cross-sectional surface of muscles, (2) better respiration and oxygen supply to sustain mechanical power generation and to compensate for the hypoxic conditions of high altitude flights, and/or (3) steady energy supply by organs providing protein substrate

for catabolism, along with fat, to sustain flight (Battley et al. 2000; Jenni et al. 2000; Klaassen 1996; Pennycuik 1998; Piersma et al. 2005). Besides exercise organs, nutritional organs (gizzard, liver) also contribute to the energy turnover, and hence the physiology of flight, by regulating food processing. While exercise organs are functional during flight, nutritional organs are atrophied during migration to reduce loading and hypertrophied only at stopover refuelling stations. It was shown that migrants have larger bony surfaces for flight muscle attachment (Calmaestra and Moreno 2000), but similar pectoral muscle and heart weights as sedentary species, except that heart size was larger in migrant Charadriiformes based on a non-phylogenetic comparative analysis (Winkler and Leisler 1992). A phylogenetic analysis based on large sample size and data for the size of multiple organs analysed in a unified approach is thus desirable for knowing how organ weights are related to migration demands.

Besides the size of specific organs, total body mass also matters in optimal migration. Body mass is the most important life-history trait, which can adaptively evolve to meet certain demands or it might constrain the realization of optimal strategies. Migration is not an exception. Body mass or drag due to mass are integral parts of functions about flight economy (Hedenström 2008; Norberg 1994; Pennycuik 2008). Therefore, body mass is expected to evolve with migration distance, although whether an increase or decrease in body mass is advantageous for long-distance migrants is still not established (Hedenström 2008). Previous studies found that body mass either decreases (Jones and Witt 2014; Kaboli et al. 2007; Sol et al. 2005) or increases (Mönkkönen 1992; Winkler and Leisler 1992) with longer migration. Furthermore, morphological adaptation to migration might be constrained by mass due to scaling laws (see e.g. Alerstam et al. 2007), and hence correction for allometry is recommended. However, several comparative studies either did not control for allometry or computed the scaling power of morphologies without accounting for the similarity due to common descent. If evolution of morphological traits is governed by selection due to migratory performance, the mass-independent scatter in morphology is expected to be functional and hence explain variation in migration distance.

Modern phylogenetic analyses of the correlates of migration distance are scarce (Bennett and Owens 2002; Leisler and Winkler 2003), in particular those based on large sample sizes, despite a long history of the topic (Fredericus 1240 cited in Lockwood et al. 1998). Whether external morphology is adapted to meet migratory demands is still questioned, while internal morphological adaptations are poorly understood because only a handful of studies addressed this question at the interspecific level.

Furthermore, although morphological adaptation to migration was subject of scientific enquiry (see references above), most of the previous comparative studies have one or more of the following limitations: (1) lack of phylogenetic control or comparison of highly contrasting sister taxa, (2) small sample sizes or narrow taxonomic ranges (few species or single families studied), (3) migration distance either scored on a two- or four-level scale with subjective thresholds or quantified by manual measurements of distribution maps, and (4) morphological traits tested in isolation instead of being tested concurrently in multiple regression models.

Everything else being equal, migration distance increases both energetic and time costs of flight (Hedenström 1993). Aerodynamic theory predicts that long-distance migrants should have high aspect ratio wings to minimize energy costs (Rayner 1988). High aspect ratio is predicted to be accompanied by either low wing loading (i.e. long wingspan) or higher wing loading (i.e. average or short wingspan) depending on whether low cost of transport (slow and economic flight) or time-efficient travel (faster, but still economic) is the currency under selection, respectively (Norberg 1990). Given that flight power is a function of pectoral muscle weight and flight aerobic capacity is enhanced by larger heart size (Bishop 1997; Rayner 1988), we predicted that exercise organ weights relative to unit mass will be positively associated with migration distance. To test these predictions rooted in aerodynamic theory, we compiled an unprecedented dataset in terms of number of species (149 European bird species), taxonomic span (50 families and 20 orders) and number of morphological traits (size of five organs, four wing size and shape parameters and body mass), which allows for generalisation. We analysed migration distance as a function of morphology by means of phylogenetic models, which constitute a powerful tool for revealing adaptations, and they are claimed suitable for revealing functional adaptations in morphology (Wainwright and Reilly 1994; Piersma et al. 2005). Passerine birds might differ from non-passerines in terms of optimal aerodynamics, which arise from the difference in basal metabolic rate between the two groups (Hedenström 1993). This is also mirrored in morphological adaptations to flight expenses (Rayner 1988). For instance, passerines have a relatively uniform and characteristic flight mode that differs from other avian taxa (Bruderer et al. 2010), and flight type might be related to the morphology of the flight apparatus (Pap et al. 2015; Wang et al. 2011). To assess whether passerines evolved a different set of functional morphology than non-passerines, we also tested our models of migration distance by restricting the analyses to the order of Passeriformes.

Materials and Methods

Specimens

Birds were either captured in the field or collected as fresh carcasses. Captures took place across Romania by using mist nets (Ecotone, Poland) or rarely traps during the breeding seasons 2009–2013. Only adult birds were considered because wing morphologies are predicted to maximize fitness in an age-specific manner (Fiedler 2005). Each bird was banded with a unique metal ring and measured for standard biometry. We took digital pictures (Nikon D80) of the left wing and the body; the wing was fully unfolded and flattened such that the leading edge was held perpendicular to the body axis. The camera was held perpendicular to the wing surface and a ruler was used as metric template. Carcasses from natural deaths (e.g. road kill, building collision, electrocution, starvation) were collected in Romania and Denmark for taxidermy. Those collected in Romania were photographed for wing architecture, while those brought to JE in Denmark contributed to the organ size data. Only carcasses in good condition were considered. Although there is spatial discrepancy in the source of wing architecture and organ weight data, we are confident that these do not affect our analyses for the following reasons. First, APM has also measured wing architecture from wing outline drawings of birds captured in Northern Europe (98 species overlap with the Romanian dataset). Those measures are strongly positively correlated with those in the present study (phylogenetic generalized least squares regression, aspect ratio: $F_{1,95} = 878.10$, $R^2 = 0.90$, $P < 0.0001$; wing loading: $F_{1,94} = 2458.00$, $R^2 = 0.96$, $P < 0.0001$). Second, in the dataset presented here, within species variance was much smaller than the among species variance (see “Results” for intraspecific repeatabilities). Third, similarly to wing morphology, it was previously shown that organ sizes are species-specific and similar between localities within the same species (Garamszegi et al. 2002; Møller et al. 2005). These findings together suggest that external and internal morphological traits are species-specific and thus suitable for multispecies comparison.

Migration Distance

Distribution maps (shape files) were retrieved from <http://www.birdlife.org/datazone/info/spcdownload> (BirdLife International and NatureServe 2012). Maps were usually restricted to longitudes between W20° and E60° (Western Palearctic), but for certain species map boundaries had to be modified according to our knowledge of the migratory behaviour of the subspecies or populations from which

morphological data were gathered (see Electronic Supplementary Material, ESM, Table S1 for modifications). Using the distribution map shape files, we calculated the centroids of the spatial polygons of breeding and wintering ranges using the ‘gCentroid’ function of the R package ‘rgeos’ (Bivand and Rundel 2013). Migration distance was then calculated as the geographic distance between the breeding and wintering centroids using a custom function written in R 3.1 (R Core Team 2015) (see Methodological details in ESM). The latitudinal centroid of the breeding range (LCB) was used as a potential ecological confounder in the analyses since northerly-breeding birds have a higher tendency to be more migratory. The species pool we study captures a great deal of variation in migration distance (range 0–8526 km).

Wing Architecture

Wing architecture comprises four traits: wingspan (m), wing area (m²), wing loading (Nm⁻²) and aspect ratio (dimensionless). These were measured based on wing pictures (see Methodological details in ESM). Wing loading (weight/wing area) gives the relative size of the wings, and aspect ratio (wingspan²/wing area) describes both the size and shape of the wing (high values indicate a narrow wing relative to its length). Our dataset covers a huge variation in body mass (range 5.6–4263.5 g, 760-fold) and wing parameters (wingspan: 0.13–1.83 m, 14-fold; wing area: 0.0042–0.61 m², 145-fold; aspect ratio: 3.72–9.14, 2.5-fold; wing loading: 10.61–195.84 Nm⁻², 18.5-fold; see ESM Table S1).

Organ Size

Numerous bird specimens were brought frozen to JE, more than 95 % of them being found dead and the remaining were shot by hunters. Danish taxidermists are required by law to record the cause of death of all specimens in a log-book. Various visceral organs, labelled as exercise (left pectoralis major muscle [subsequently pectoral muscle], left supracoracoid muscle, heart) or nutritional organs (liver and gizzard) were removed during post-mortem examination and weighed by JE on a precision balance to the nearest 1 mg. Only birds with undamaged organs were considered, while storage caused negligible noise in organ masses (Møller et al. 2005). Data were recorded blindly with respect to the tested hypothesis. Our dataset covers a huge variation in organ mass (gizzard: 0.15–57.22 g, 381-fold; liver: 0.28–68.18 g, 243-fold; pectoral muscle: 0.35–182.79 g, 522-fold; supracoracoid muscle: 0.03–15.15 g, 505-fold; heart: 0.08–27.42 g, 343-fold; see ESM Table S1).

Statistical Analyses

All statistical analyses were conducted as implemented in R 3.1 (R Core Team 2015). To assess whether morphological traits are species-specific and thus suitable for multispecies comparison (Møller and Birkhead 1994), we tested the repeatability of these traits by assessing the importance of among-species compared to within-species variance (function ‘ICCest’ from R package ‘ICC’; Wolak et al. 2011). Since variance is dependent on sample size, the estimation of repeatability in comparative studies with unbalanced sample sizes across species is not equivocal. To cope with this problem, as a first step, species with only one individual sampled were excluded. Subsequently, we randomly picked two values out of the total number of measurements per species for the remaining species and partitioned variance to within- and among-group components. This was iterated 1000 times, so repeatabilities are the average of the 1000 repeatability estimates. Repeatability was tested using raw, non-transformed values.

Subsequently, we investigated by means of regression analyses how avian external and internal morphological traits (1) covary with each other, (2) scale to body mass and (3) explain migration distance. However, in regressions across multiple species the error terms of different species are correlated such that covariance is higher among closely related species due to longer shared phylogenetic history. Therefore, we developed phylogenetically informed models that account for the dependence of species by incorporating a matrix of covariances among species based on their phylogenetic relationships (Martins and Hansen 1997; Pagel 1997, 1999), and we estimated the importance of phylogenetic corrections (Freckleton et al. 2002). These analyses were based on the dated phylogeny published by Thuiller et al. (2011). Because five of our species were missing from this tree, our original sample size of 154 species dropped to 149. We used phylogenetic generalized least squares (PGLS) models as implemented in the ‘pgls’ function of the R package ‘caper’ (Orme et al. 2011). The phylogenetic signal Pagel’s λ (Pagel 1997, 1999) was estimated by maximum likelihood in each model. Strong and weak signal (i.e. λ approaches 1 and 0, respectively) indicates that evolution conforms to or deviates from the Brownian motion model, respectively. Three transformations were adopted prior to the analyses: (1) body mass and all morphological traits were log₁₀-transformed (LCB and migration distance were unaltered because raw values provided better fit to the distribution requirement of model residuals), (2) all morphological trait values were adjusted to unit body mass to rule out a scaling effect, and (3) all fixed effects were standardized to a mean of zero and a SD of one in order to avoid the large difference between the

intervals covered by different traits (cf. wing loading with body mass in ESM Table S1).

First, the pairwise relationships between the nine morphological characters as well as body mass was assessed by bivariate PGLS models with one trait as dependent and the rest sequentially set as a single fixed term. Second, to estimate the scaling of morphological characters to body mass, we used phylogenetically informed reduced major axis (PRMA) regressions ('*phyl.RMA*' function from R package '*phytools*'; Revell 2012) because accounting for dependence due to common descent is highly recommended in scaling studies (Harvey 2000). Scaling is expressed as a power function of the form $Y = a \times \text{mass}^b$, where Y is one of the morphological variables. With log-transformation the function becomes $\log(Y) = \log(a) + b \times \log(\text{mass})$, where a is the PRMA regression coefficient and b is the PRMA regression slope. Third, we fitted three sets of PGLS models with migratory flight distance as response variable and the following fixed effect formula: (1) all internal morphological traits + \log_{10} body mass + LCB; (2) all external morphological traits + \log_{10} body mass + LCB; and (3) all internal and external morphological traits + \log_{10} body mass + LCB (hereafter referred to as 'organ size', 'wing morphology' and 'organ and wing' models, respectively). These three sets of multiple regression models were reduced to minimal adequate models by a backward stepwise elimination procedure, discarding the least significant predictor at each step until only significant predictors remained. Using the information-theoretic approach and multi-model averaging yielded qualitatively similar results (not shown). We did not model second-order interactions between morphological traits since (1) this would enormously increase the number of parameters to be estimated leading to undue overfitting, and (2) the correlation between main effects and interaction terms would imply a serious multicollinearity issue.

Because morphological traits intercorrelate even after adjustment for body mass (see "Results"), we verified whether there is a multicollinearity problem in the models by computing the variance inflation factors (VIFs) within each MAM of the three model sets and found that values were all below the more conservative VIF <5 threshold. Therefore, multicollinearity does not bias our results. We also employed two filtering. First, we repeated the above three model sets by only using Passeriformes (84 out of 149 species, 56.4 %) to assess the robustness of the results to difference between passerines and non-passerines. Second, we repeated the above three model sets by excluding resident species with the filtering criterion of migration distance >0 km (127 out of 149 species, 85.2 %) to assess whether the morphological predictors of migration distance could arise due to the disparity between resident and migratory species subsets.

Results

Repeatability and Scaling

Each organ size and wing morphology trait was highly repeatable, and repeatability values, expressed as intra-class correlation coefficient (ICC), had a narrow confidence interval, which together show that these traits are highly species-specific (gizzard: $ICC = 0.93$, 95 % CI 0.90–0.96; liver: $ICC = 0.78$, 95 % CI 0.69–0.84; pectoral muscle: $ICC = 0.76$, 95 % CI 0.64–0.84; supracoracoid muscle: $ICC = 0.75$, 95 % CI 0.64–0.83; heart: $ICC = 0.92$, 95 % CI 0.89–0.95; wingspan: $ICC = 0.99$, 95 % CI 0.99–0.99; wing area: $ICC = 0.98$, 95 % CI 0.97–0.99; aspect ratio: $ICC = 0.91$, 95 % CI 0.88–0.94; note that wing loading has identical repeatability to that of wing area; for organ weights see also Garamszegi et al. 2002; Møller et al. 2005). Hence, species are confidently characterised in our dataset even if they are represented by only two individuals. Therefore, we included species irrespective of samples size per morphological trait in the analyses.

The scaling exponents of the nine morphological traits are shown in Table 1. We derived the mass-adjusted morphological trait values using the equation mass-adjusted trait = original trait/mass^{*b*}, where b is the scaling exponent (i.e. PRMA slope). These mass-independent morphological trait values were used in the subsequent analyses.

Covariation of Morphological Traits

External and internal morphological traits were significantly associated (all traits \log_{10} -transformed but not corrected for body mass, all $P \leq 0.02$; not shown). These associations probably stem from the body mass-dependence of morphological traits. However, a large number of

Table 1 Scaling of organ size and wing morphology traits estimated by phylogenetic reduced major axis (PRMA) regression

Morphological trait	<i>n</i>	<i>a</i>	<i>b</i>	R^2	Pagel's λ
Gizzard	107	−1.65	1.02	0.79	0.96
Liver	112	−1.45	0.94	0.85	0.91
Pectoral muscle	90	−1.24	1.01	0.89	0.92
Supracoracoid muscle	88	−2.06	0.98	0.85	1.00
Heart	112	−1.97	0.95	0.92	0.94
Wingspan	149	0.87	0.36	0.84	0.97
Wing area	149	1.04	0.69	0.85	0.96
Aspect ratio	149	0.43	0.14	0.04	0.96
Wing loading	149	0.59	0.45	0.65	0.96

PRMA coefficient, a , and slope, b , are reported together with the coefficient of determination, R^2 , and phylogenetic signal, Pagel's λ . For details, see "Materials and Methods"

relationships remained significant after traits were adjusted to unit body mass (ESM Table S2).

Migration Distance

The MAMs of each of the three multiple regression PGLS model sets contain LCB as a positive predictor of distance between breeding and wintering grounds with northerly-breeding species migrating longer distances for all species or passerines only (Tables 2, 3, 4). Body mass was a significant negative predictor of migration distance in the ‘organ size’ model set and when the analysis was based on the entire species pool, while in other models it was dropped during model reduction to MAMs (Tables 2, 3, 4).

In the ‘organ size’ model set (Table 2), mass-adjusted heart weight was significantly inversely related to migration distance in the full models based on both the entire species pool and on passerines only. However, it was dropped during model simplification to MAMs with other organ size traits. Therefore, none of the MAMs included the size of any organ to explain variation in migration distance among species (Table 2). Similarly, the size of

neither organ was retained when only the passerines were considered (Table 2). The MAM of the entire species pool fitted significantly better the data than the null model, which modelled only the intercept and did not include any organ size trait (AIC = 683.48 and 670.97 for null model and MAM, respectively; likelihood ratio test, LRT, of null model vs. MAM: LR = 16.51, df = 3, 5, P = 0.0003). The MAM based on only migratory species is the null model, since all morphological traits and the confounding LCB were rejected during model reduction.

In the ‘wing morphology’ model set (Table 3), wing loading was the only significant predictor of migration distance in the full model involving all studied species, where species making longer journeys having lower loadings. After model simplification to MAMs, however, aspect ratio was also retained besides wing loading when the analysis was based on the entire species pool. Species that migrate farther have significantly higher aspect ratio. When the analysis was restricted to passerines, both wing loading and aspect ratio were rejected, and wingspan and wing area became significant explanatory variables. Passerines that migrate farther have longer wingspan and smaller wing

Table 2 ‘Organ size’ model set: multiple regression PGLS model of migration distance in relation to log₁₀ body mass, latitudinal centroid of the breeding range (LCB) and all the organ size traits adjusted for body mass

Full model				MAM			
Predictors	β (SE)	t	P	Predictors	β (SE)	t	P
<i>(a) All species</i>				<i>(a) All species</i>			
<i>n</i> = 83, Pagel’s λ = 0.98				<i>n</i> = 149, Pagel’s λ = 0.80			
Intercept	3.39 (1.41)	2.41	0.019	Intercept	3.75 (1.10)	3.39	<0.001
Log ₁₀ body mass	−0.84 (0.49)	1.73	0.088	Log ₁₀ body mass	−1.26 (0.37)	3.39	<0.001
LCB	0.43 (0.22)	1.94	0.056	LCB	0.45 (0.17)	2.67	0.009
Gizzard	−0.32 (0.31)	1.02	0.313				
Liver	0.50 (0.36)	1.38	0.173				
Pectoral muscle	0.55 (0.35)	1.59	0.115				
Supracoracoid muscle	−0.61 (0.45)	1.36	0.179				
Heart	−0.77 (0.35)	2.22	0.03				
<i>(b) Only passerine species</i>				<i>(b) Only passerine species</i>			
<i>n</i> = 55, Pagel’s λ = 0.99				<i>n</i> = 84, Pagel’s λ = 0.86			
Intercept	1.80 (0.93)	1.92	0.06	Intercept	2.11 (0.92)	2.31	0.024
Log ₁₀ body mass	−0.47 (0.38)	1.22	0.229	LCB	0.61 (0.19)	3.17	0.002
LCB	0.34 (0.25)	1.36	0.182				
Gizzard	−0.59 (0.39)	1.52	0.135				
Liver	0.82 (0.43)	1.92	0.062				
Pectoral muscle	0.75 (0.41)	1.83	0.074				
Supracoracoid muscle	−0.33 (0.46)	0.73	0.468				
Heart	−0.59 (0.29)	2.02	0.049				

Both the full model and the derived minimal adequate model (MAM) are shown for analyses based on either (a) the entire species pool or (b) restricted to passerines. Significant relationships are highlighted in bold

Table 3 ‘Wing morphology’ model set: multiple regression PGLS model of migration distance in relation to \log_{10} body mass, latitudinal centroid of the breeding range (LCB) and all wing morphology traits adjusted for body mass

Full model				MAM			
Predictors	β (SE)	t	P	Predictors	β (SE)	t	P
(a) <i>All species</i>				$n = 149$, Pagel’s $\lambda = 0.76$			
Intercept	3.47 (0.92)	3.76	<0.001	Intercept	3.20 (0.87)	3.67	<0.001
\log_{10} body mass	−0.92 (0.66)	1.39	0.167	LCB	0.42 (0.15)	2.71	0.008
LCB	0.44 (0.15)	2.90	0.004	Aspect ratio	1.67 (0.22)	7.67	< 0.001
Wingspan	0.91 (1.75)	0.52	0.605	Wing loading	−0.69 (0.24)	2.88	0.005
Wing area	−1.46 (1.28)	1.14	0.257				
Aspect ratio	1.15 (0.90)	1.27	0.206				
Wing loading	−1.34 (0.60)	2.23	0.028				
(b) <i>Only passerine species</i>				$n = 84$, Pagel’s $\lambda = 0.86$			
Intercept	2.57 (0.76)	3.37	0.001	Intercept	2.49 (0.78)	3.20	0.002
\log_{10} body mass	−0.98 (0.91)	1.08	0.286	LCB	0.59 (0.17)	3.57	< 0.001
LCB	0.58 (0.17)	3.32	0.001	Wingspan	2.74 (0.51)	5.33	< 0.001
Wingspan	2.84 (3.35)	0.85	0.398	Wing area	−1.67 (0.48)	3.51	< 0.001
Wing area	−2.87 (2.01)	1.43	0.157				
Aspect ratio	−0.16 (2.23)	0.07	0.943				
Wing loading	−1.24 (1.20)	1.04	0.303				

Both the full model and the derived minimal adequate model (MAM) are shown for analyses based on either (a) the entire species pool or (b) restricted to passerines. Significant relationships are highlighted in bold

area (Table 3). The MAM of the entire species pool fitted significantly better the data than the null model, which modelled only the intercept and did not include any wing morphology trait (AIC = 683.48 and 634.86 for null model and MAM, respectively; LRT of null model vs. MAM: LR = 54.62, $df = 3, 6, P < 0.0001$). The MAM based on only migratory species contained the same significant predictors as the MAM without restriction to migrants (n species = 127, Pagel’s $\lambda = 0.74$; LCB: $\beta \pm SE = 0.45 \pm 0.17, t = 2.63, P = 0.01$; mass-adjusted aspect ratio: $\beta \pm SE = 1.58 \pm 0.26, t = 6.14, P < 0.0001$; mass-adjusted wing loading: $\beta \pm SE = -0.76 \pm 0.26, t = 2.86, P = 0.005$).

In the ‘organ and wing’ model set (Table 4), mass-adjusted heart mass was negatively related to migration distance in the full models of all species and passerines only, while mass-adjusted aspect ratio was positively related to migration distance in the full model involving all species. After minimization to MAMs, these two traits remained significantly associated with migration distance; species that cover longer distances have smaller relative heart weight and higher aspect ratio (Table 4; Fig. 1). The MAM of the entire species pool fitted significantly better the data than the null model, which modelled only the intercept and did not include any organ size or wing morphology trait (AIC = 502.65 and 464.13 for null model and MAM, respectively; LRT of null model vs. MAM: LR = 44.52,

$df = 3, 6, P < 0.0001$). The MAM based on only migratory species contained the same significant predictors as the MAM without restriction to migrants (n species = 92, Pagel’s $\lambda = 0.88$; LCB: $\beta \pm SE = 0.44 \pm 0.19, t = 2.35, P = 0.02$; mass-adjusted heart weight: $\beta \pm SE = -0.62 \pm 0.27, t = 2.30, P = 0.02$; mass-adjusted aspect ratio: $\beta \pm SE = 1.88 \pm 0.31, t = 5.99, P < 0.0001$).

To verify the robustness of our results, we conducted two sets of sensitivity analyses. First, because flapping or soaring flight style might favour different morphological traits in long-distance migrants, we tested whether exclusion of soaring birds (9 species; flight style label ‘2a’ in Bruderer et al. 2010) altered the results, but found qualitatively similar results (see ESM Tables S3–S5). Second, because organ sizes can considerably change as a function of migratory stage, we excluded all individuals with unknown collection date or collected during spring or fall migratory periods and once again we found qualitatively unchanged results (see ESM Tables S6 and S7).

Discussion

The prevailing view on the evolution of the flight apparatus suggests that flight-related morphology is evolutionarily labile (Piersma et al. 2005) and mirrors better the habitat-

Table 4 ‘Organ and wing’ model set: multiple regression PGLS model of migration distance in relation to \log_{10} body mass, latitudinal centroid of the breeding range (LCB) and all organ size and wing morphology traits adjusted for body mass

Full model				MAM			
Predictors	β (SE)	<i>t</i>	<i>P</i>	Predictors	β (SE)	<i>t</i>	<i>P</i>
(a) <i>All species</i> <i>n</i> = 83, Pagel’s λ = 0.96				<i>n</i> = 112, Pagel’s λ = 0.87			
Intercept	2.51 (1.16)	2.16	0.034	Intercept	2.84 (0.96)	2.96	0.004
Log ₁₀ body mass	0.90 (0.95)	0.95	0.346	LCB	0.39 (0.17)	2.33	0.021
LCB	0.30 (0.20)	1.52	0.133	Heart	−0.54 (0.23)	2.34	0.021
Gizzard	−0.17 (0.27)	0.64	0.521	Aspect ratio	1.83 (0.26)	7.14	< 0.001
Liver	0.44 (0.32)	1.36	0.179				
Pectoral muscle	0.10 (0.35)	0.30	0.768				
Supracoracoid muscle	0.06 (0.41)	0.15	0.883				
Heart	−0.85 (0.33)	2.58	0.012				
Wingspan	−2.50 (2.35)	1.06	0.292				
Wing area	1.55 (1.90)	0.82	0.417				
Aspect ratio	3.20 (1.28)	2.50	0.015				
Wing loading	−1.07 (0.85)	1.26	0.213				
(b) <i>Only passerine species</i> <i>n</i> = 55, Pagel’s λ = 0.91				<i>n</i> = 67, Pagel’s λ = 0.83			
Intercept	2.38 (0.72)	3.32	0.002	Intercept	2.12 (0.74)	2.84	0.006
Log ₁₀ body mass	0.70 (0.83)	0.84	0.403	LCB	0.56 (0.21)	2.70	0.009
LCB	0.25 (0.24)	1.04	0.306	Heart	−0.63 (0.27)	2.36	0.021
Gizzard	−0.29 (0.34)	0.85	0.40	Aspect ratio	1.74 (0.31)	5.57	< 0.001
Liver	0.65 (0.38)	1.69	0.098				
Pectoral muscle	−0.14 (0.42)	0.33	0.74				
Supracoracoid muscle	0.45 (0.42)	1.07	0.292				
Heart	−0.71 (0.30)	2.32	0.025				
Wingspan	−1.19 (3.37)	0.35	0.725				
Wing area	0.82 (1.87)	0.44	0.662				
Aspect ratio	2.63 (2.39)	1.10	0.277				
Wing loading	−0.62 (1.29)	0.48	0.635				

Both the full model and the derived minimal adequate model (MAM) are shown for analyses based on either (a) the entire species pool or (b) restricted to passerines. Significant relationships are highlighted in bold

specific feeding behaviour and related flight style than migratory behaviour (e.g. Rayner 1988; Norberg 1990; Leisler and Winkler 2003). By contrast, we found that such traits are conservative as evidenced by a strong phylogenetic signal. Despite this phylogenetic constraint, a few traits were significant predictors of migration distance across an ecologically diverse array of species studied.

Body mass was not a consistent predictor of migration distance being retained only in the organ size model set based on the entire species pool. Our findings thus suggest that the aerodynamic advantages of neither small nor large body mass drive the evolution of body mass to meet the challenges imposed by long-distance migratory flights. The weak association between body mass and migration distance is probably the mere consequence of several selective forces governing the evolution of body size (Winkler and

Leisler 1992), which is evidenced by the wide scatter of body masses even among long-distance migrants (from swallows to large raptors). Because the weight of visceral organs has a considerable contribution to body mass, and because we measured them mostly outside the migratory period, future studies analysing organ sizes measured on migratory birds could clarify this question.

Aspect ratio was proposed by many as a key adaptation to migration (see “Introduction”; but see Rayner 1990). The single stringent test of this hypothesis was conducted by Lockwood et al. (1998), who used 27 species pairs to control for phylogenetic effects and showed that birds categorised as migrants had higher aspect ratio than residents. We extended this analysis by using 149 species, modelling migration distance as a continuous variable and using multiple regression by considering many

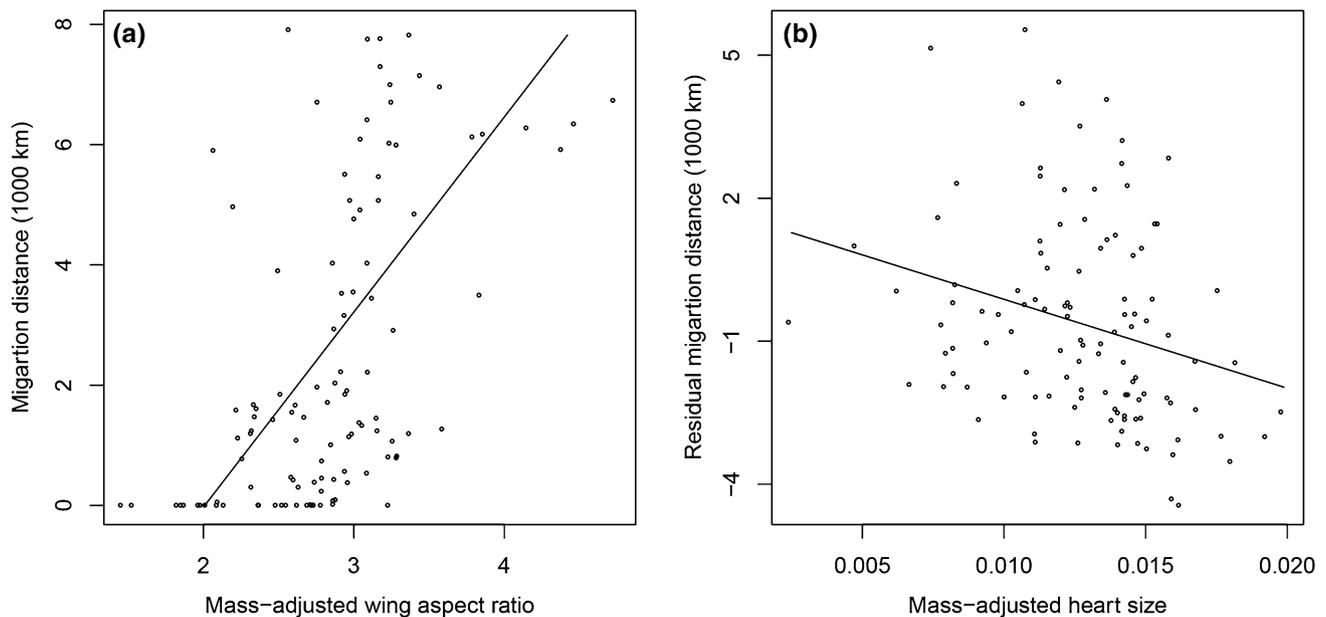


Fig. 1 Migration distance as a function of **a** mass-adjusted wing aspect ratio and **b** mass-adjusted heart mass. The graphs are drawn based on the MAM in Table 4a. On panel (b), residual migration distance reflects migration distance corrected for LCB and aspect ratio

morphological traits in addition to aspect ratio. Our findings corroborate that of Lockwood et al. (1998). Previous comparative studies did not find a correlation between wing loading and migration distance (Marchetti et al. 1995; Winkler and Leisler 1992), but we did when considering only external morphological traits and the entire species pool. This study is the first (1) to show a strong relationship between migration distance and heart weight and (2) to simultaneously analyse many morphological traits by taking phylogenetic and scaling effects into account.

The amount of work done to maintain level flight is a function of the magnitude of the net lift (i.e. lift-to-drag ratio), and the size and shape of the wing is decisive in this respect (Norberg 1990; Pennycuick 2008). Selection might not only minimize workload (i.e. energy or power per unit distance, also coined energy-efficiency or cost of transportation), but time per unit distance flown as well (i.e. time-efficiency). The two currencies are related to each other via flight velocity and because long travels assimilate more energy (Hedenström 1993). It is still unresolved which currency is more important for migrants (for energy, see Rayner 1988; Norberg 1994; for time, see Hedenström 1993, 2008). Lindhe Norberg (2002) argued that high aspect ratio combined with short wings (i.e. high wing loading) is favourable for long commuting flight because it ensures fast but still economic flight. Rayner (1988) used principal component analysis to obtain axes that correspond to size, wing loading and aspect ratio, and found that maximum range power (i.e. power needed to fly maximum range with unit energy) increases with larger size and

higher wing loading and decreases with higher aspect ratio, and migrants have high aspect ratio and low to medium wing loading. We reconsidered these associations by controlling for scaling and phylogenetic effects and found that long-distance migrants might reduce the maximum range power via higher aspect ratio, while wing loading has limited effects. In the model restricted to passerines, aspect ratio was discarded from the model, while wingspan was longer and wing area smaller with increasing migration distance. Note however, that long wingspan in combination with small wing area is characteristic for high aspect ratio wings. Therefore, high aspect ratio seems to be a general adaptation to long-distance migration in birds.

High aspect ratio is acknowledged for reducing the cost of transportation (i.e. maximization of flight range per unit energy) because it decreases the induced and profile drag (at low-to-medium and high airspeed, respectively), better shedding of wingtip vortices, and better glide ratio (sinking per horizontal speed) (Norberg 1990, 1994; Pennycuick 2008; Rayner 1988; Shyy et al. 2008; Withers 1981). Note that high aspect ratio might increase wing loading owing to the narrow wing mean chord (Norberg 1990; Alerstam et al. 2007; this study) and ultimately flight energetics (Rayner 1988), inasmuch as wingspan does not change. Depending on model set, wing loading either decreased or was not related to migration distance indicating that wingspan increased in parallel with aspect ratio, which prevented an elevated wing loading. The positive correlation of aspect ratio with wingspan, but lack of correlation with wing area, and the only subtle decrease of wing area

with migration distance lend support to this assertion (ESM Table S2). The combination of high aspect ratio with low-to-moderate wing loading is thought to be highly energy-efficient (Norberg 1990). Taken together, our results suggest that flight economy has selective priority over time-efficiency.

Contrary to our predictions, migration distance was not associated with flight muscle sizes and has a strong inverse relationship with heart weight. The supracoracoid muscle may function more in wing strokes related to diverse flight modes and in turn not being shaped by selection due to migratory flight. In contrast, pectoral muscle and heart weight directly determine the power available for flight (Bishop 1997; Rayner 1988). Further tests are required to clarify this questions, for instance, by collecting flight muscle size data from individuals belonging to several bird species that are prior to take on wings (exhibit zugunruhe and associated hypertrophied muscles) or are en route (exhibit either hypertrophied or atrophied muscles depending on how long they migrated before or on refuelling state).

Alternatively, organ weight of long-distance migrants might mirror the energetic exigencies that these birds face. Evidence from avian comparative studies (e.g. Wiersma et al. 2012) and mammalian artificial selection lines (Konarzewski and Diamond 1995) show that heart tissue is energetically expensive. Furthermore, both pectoral muscle and heart weight are considered indicators of aerobic costs during extensive locomotion (Bishop 1997; Klaassen 1996), which is supported by species with energetically expensive flight mode possessing larger relative flight muscle and heart weight in order to produce more power and to meet the aerobic scope (Bishop 1997; Norberg 1990). The average-sized flight muscles of long-distance migrants do not demand a large heart because heart size is optimized to satisfy the oxygen demand of exercise tissues (Bishop and Butler 1995). On the other hand, accepting the higher aspect ratio coupled with moderate wing loading as energy-saving adaptations in long-distance migrants, this might permit the evolutionary shrinkage of energetically costly exercise organs. A similar case has been reported for swordtail fish; species with longer sexually selected sword had higher swimming velocity and smaller heart mass (Oufiero et al. 2014). It should be noted, however, that the organ sizes presented here reflect the baseline levels and are not related to the size adjustments in the pre- or post-migratory periods. Great knots *Calidris tenuirostris* substantially reduce their organ sizes, except brain and lung, during a 5400 km flight (Battley et al. 2000). Therefore, the energetic hypothesis proposed above should be tested with comparative data for the relationship between migration distance and the magnitude of change in organ sizes between pre- and post-migratory periods.

Given the high demands of long-distance migration and a vast array of external and internal morphological traits expected to be related to these demands, it was proposed that a ‘migratory syndrome’ should exist (Dingle 2006; Piersma et al. 2005). The existence of syndromes implies that the traits that integrate into a syndrome are correlated by being either synergistic or antagonistic (Dingle 2006). However, the covariance of internal and external morphological traits thought to be part of the flight apparatus was not scrutinized by accounting for scaling and phylogenetic effects. Two recent reviews concluded that an integrated migratory syndrome is unlikely to exist because there are multiple solutions to the same migratory problem, and, therefore, only a subset of the allegedly migratory trait requirements are found per species (Dingle 2006; Piersma et al. 2005). Our results agree with this viewpoint, since only a few traits were strong predictors of migration distance despite the fact that several morphological traits correlate with each other.

To summarize, we showed that some morphological traits are likely adaptations to long-distance migration and related flight costs. Moreover, small-sized passerines seemingly are not exempt from this selection pressure. Therefore, we disagree with others (see also Voelker 2001), who suggested that migratory flight performance is negligible particularly in small birds and at best leads to subtle differences in morphology (Leisler and Winkler 2003; Rayner 1988; Winkler and Leisler 1992). Combining the results about the predictors of migration distance and covariance of morphological traits suggests that selection primarily optimizes the preservation of energy assets during long travels in order to increase flight range per unit energy. By virtue of their high aspect ratio that conserves energy and moderate wing loading that generates sufficient lift, migrants might not be subject to strong selection for large flight muscle and aerobic capacity. We encourage the collection of a considerable amount of new morphological data from several other bird species because questions like how flight style affects functional morphology of migrants and the scaling rules of these traits, and how organ sizes are adjusted during the migratory season deserve future comparative studies.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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Light enough to travel or wise enough to stay? Brain size evolution and migratory behavior in birds

Orsolya Vincze^{1,2,3}

¹MTA-DE “Lendület” Behavioural Ecology Research Group, Department of Evolutionary Zoology and Human Biology, University of Debrecen, Debrecen H-4032, Hungary

²Evolutionary Ecology Group, Hungarian Department of Biology and Ecology, Babeş-Bolyai University, Cluj-Napoca 400006, Romania

³E-mail: orsolya.vincze@vocs.unideb.hu

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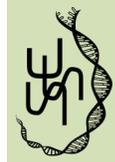
Brain size relative to body size is smaller in migratory than in nonmigratory birds. Two mutually nonexclusive hypotheses had been proposed to explain this association. On the one hand, the “energetic trade-off hypothesis” claims that migratory species were selected to have smaller brains because of the interplay between neural tissue volume and migratory flight. On the other hand, the “behavioral flexibility hypothesis” argues that resident species are selected to have higher cognitive capacities, and therefore larger brains, to enable survival in harsh winters, or to deal with environmental seasonality. Here, I test the validity and setting of these two hypotheses using 1466 globally distributed bird species. First, I show that the negative association between migration distance and relative brain size is very robust across species and phylogeny. Second, I provide strong support for the energetic trade-off hypothesis, by showing the validity of the trade-off among long-distance migratory species alone. Third, using resident and short-distance migratory species, I demonstrate that environmental harshness is associated with enlarged relative brain size, therefore arguably better cognition. My study provides the strongest comparative support to date for both the energetic trade-off and the behavioral flexibility hypotheses, and highlights that both mechanisms contribute to brain size evolution, but on different ends of the migratory spectrum.

KEY WORDS: Behavioral flexibility, cognition, energy trade-off, innovation, migration.

Brain size relative to body size has long been considered a major determinant of the cognitive abilities of a given individual, or species (Sol 2009). For instance, large relative brain size has been linked to a wide range of benefits, including increased survival, adaptability to novel environments, innovation propensity, variability of habitats occupied, invasiveness, and sociability (Lefebvre et al. 2004; Lefebvre and Sol 2008; Sol 2009; Sol et al. 2007, 2010; Lefebvre 2013). Nevertheless, in spite of the multifaceted benefits conferred by a large brain (relative to body size), there is a downside: high metabolic cost (Isler and van Schaik 2009; Sol 2009). The brain is one of the most energetically expensive organs in the body, consuming up to 10 times more energy per unit mass than skeletal muscle (Isler and van Schaik 2006, 2009).

Therefore, relative brain size in a given species should reflect a careful balance between costs and benefits; the evolutionary optimum should be the size that maximizes survival and reproductive success as a function of species ecology, life history, and behavior (Sol et al. 2010).

One major ecological constraint on relative brain size across flying homothermic vertebrates is distance travelled during migration (Winkler et al. 2004). This association has repeatedly been demonstrated at the species level in birds (e.g., Winkler et al. 2004; Sol et al. 2005; Vincze et al. 2015), and bats (McGuire and Ratcliffe 2011), as well as at the subspecies level in birds (Cristof et al. 2003; Pravosudov et al. 2007; Fuchs et al. 2015). The correlation is very robust and, in all cases, relative total brain



weight decreases with increasing migration distance (Cristol et al. 2003; Winkler et al. 2004; Sol et al. 2005; Pravosudov et al. 2007; McGuire and Ratcliffe 2011; Vincze et al. 2015). The two hypotheses proposed to explain this association are, by definition, explaining variation on two different ends of the migratory distance spectrum (i.e., residents vs. long-distance migrants). The energetic trade-off hypothesis builds upon the metabolic costs of migration, and that of developing and sustaining neural tissues, suggesting energetic conflict between these two demands (Winkler et al. 2004; McGuire and Ratcliffe 2011). Migration is one of the most energetically challenging periods in a bird's life: bar-tailed Godwits (*Limosa lapponica*), for example, cover 11,000 km in a single nonstop flight (Gill et al. 2009). Such strenuous movements are often on the edge of avian physiological endurance and necessitate a range of adaptations to make the journey possible (Hedenström 2010). For instance, we know that body mass is often doubled during the premigratory fattening process to support the energetic needs of the journey (Newton 2008), while almost all organs undergo significant size reduction prior to migration to minimize the metabolic cost of transport (Piersma and Lindström 1997; Battley et al. 2000). An extensive study on the morphological adaptations to migration in birds found that heart size, the most calorie-hungry structure in the body, is relatively smaller in long-distance migrants (Vágási et al. 2016), corroborating negative selection on energetically expensive organ sizes. Given the energetic cost of flight, long-distance migration may compromise a bird's ability to support the high metabolic cost of a large brain. Thus, the energetic trade-off hypothesis predicts directional selection that favors smaller relative brain size with increasing migration distance.

In contrast, the behavioral flexibility hypothesis assumes a positive directional selection on relative brain size in resident birds (Winkler et al. 2004) instead of negative selection in migrants. Resident bird species often experience strong spatial and temporal fluctuations in their environments, and therefore tend to rely more heavily on novel food sources, exploited through innovations and learning (Sol et al. 2005; Aplin et al. 2013). Classical examples of such behaviors are the “milk bottle” innovation in Blue Tits (*Cyanistes caeruleus*, Aplin et al. 2013), or Great Tits (*Parus major*) preying on hibernating bats (Estók et al. 2009). Such behaviors usually only happen under environmental conditions (e.g., harsh winters) that limit normal food sources (Estók et al. 2009); this also highlights the importance of innovation in seasonal and harsh environments. Indeed, innovation propensity and associated relative brain size (Timmermans et al. 2000; Reader and Laland 2002) are both highest in resident species and lowest in long-distance migrants (Sol et al. 2005). Nonetheless, whether higher degrees of innovation in resident species reflects necessity, or their capacity, has yet to be determined. To attempt to address this, a reformulation of the behavioral flexibility hypothesis by inverting

causalities was coined the “migratory precursor hypothesis” (Sol et al. 2005). The elevated cognitive capacity of large-brained birds would enable them to be residents, while small-brained species are forced to migrate. Irrespective of causality, higher cognitive needs, especially if innovative behavior is socially transmitted (Aplin et al. 2013), may represent one plausible explanation for the larger relative brain sizes of resident bird species and might represent a coping mechanism to harsh or seasonal environments.

Exploring the relationship between relative brain size and the environmental harshness, or variability, experienced by resident birds in different climatic zones, or latitudes, could provide a strong test of the behavioral flexibility/migratory precursor hypothesis (Winkler et al. 2004). Studies investigating the behavioral flexibility hypothesis to date are, however, scant and results are contradictory (Schuck-Paim 2008). In neotropical parrots, climate variability was shown to be positively associated with relative brain size (Schuck-Paim 2008), which provides some intraspecific support for the behavioral flexibility hypothesis. Moreover, elevated winter harshness and the associated increased requirement for food-caching is correlated with enlargement of brain regions responsible for spatial memory in different black-capped chickadee (*Poecile atricapillus*) populations (Roth and Pravosudov 2009; Roth et al. 2011). There is thus some evidence that the environmental harshness and fluctuation influence brain evolution and functionality; however, which aspects of the environment are most important in this respect, and in what settings do selective forces act, remain unanswered. By extending the geographical and taxonomic coverage of previous studies, and by testing how different environments experienced by species with similar migratory behaviors result in relative brain size differentiation could provide potential answers to these questions.

In this study, I test separately the validity of the energetic trade-off and behavioral flexibility hypotheses, and explore the nature of the negative correlation between relative brain size and migratory behavior in birds. First using data from the literature, I assess the generality of this negative association on the basis of an extensive list of bird species ($n = 1466$), across a very wide body size (2.7 g to 44 kg) and taxonomic range, encompassing ratites to passerines. Second, using migration measured on a continuous scale (0–13,063 km) and species with migration distance >0 km (i.e., excluding residents), I test the validity of the energetic trade-off hypothesis. This hypothesis will gain support if there is a negative association between migration distance and relative brain size, and the effect will be strongest among long-distance migratory birds. Third, using only resident species ($n = 937$), across a tropical-to-arctic distributional spectrum, I test the validity of the behavioral flexibility hypothesis. In this final case, I use winter minimum temperature, seasonality of ambient temperature, and wintering latitude to test which one of these factors best predicts the relative brain size of resident birds. The behavioral flexibility

hypothesis will be supported in cases where relative brain size increases with latitude or seasonality, or decreases with increasing winter minimum temperature; note that the latter ought to have the strongest effect when the nature of this association is defined by environmental harshness. My study thus provides the first broad and fine-scale mutual test for the behavioral flexibility and energy trade-off hypotheses, to explore how these mechanisms shape the evolution of avian brains.

Materials and Methods

BRAIN AND BODY SIZE

I extracted brain and body weight data from Iwaniuk and Nelson (2003), a primary dataset listing the endocranial volumes of a wide range of birds. Endocranial volume is a highly reliable method to measure brain size both across, and within, species (Iwaniuk and Nelson 2002). Brain mass was then obtained by multiplying reported endocranial volumes by the density of fresh brain tissue, 1.036 g/mL (Iwaniuk and Nelson 2003), and the dataset was then further modified by calculating a single mean for species for which data were originally reported at the subspecies level (e.g., *Platycercus elegans elegans* and *P. e. flaveolus*), or using two synonymous names (e.g., *Esacus magnirostris* and *E. neglectus*). In these cases, species means were calculated as the weighted arithmetic mean of separate measurements where weights were represented by the number of specimens measured in each case.

Given that larger-bodied species have larger brains, body mass needs to be controlled for when comparing brain sizes across species (Lashley 1949). Body mass was therefore included in each of the models presented here, as brain size relative to body size is a measure that reflects the surplus of neural tissue versus the amount required for basic bodily functions (Lashley 1949), and is associated with a range of cognitive traits across species (see above).

MIGRATION DISTANCE

Distribution maps (shape files) for each species were downloaded from <http://www.birdlife.org/datazone/info/spcdownload> (BirdLife International and NatureServe 2014), and the geometric centroid of the spatial polygon of breeding (breeding and resident) and wintering (wintering and resident) ranges were calculated using the “gCentroid” function in the R package “rgeos” (Bivand and Rundel 2013). Migration distance was calculated as the geographic distance between breeding and wintering centroids using a custom function written in R (Vágási et al. 2016). “Migratory distance” thus denotes the average distance travelled by a given species during migration. Additionally, I extracted the latitude of the nonbreeding centroids, and calculated absolute values (thereafter, “nonbreeding latitude”).

ENVIRONMENTAL HARSHNESS AND SEASONALITY

I extracted ambient temperature data from the University of East Anglia Climate Research Unit database (CRU, <http://www.cru.uea.ac.uk/>; ver. 3.10.01; Mitchell and Jones 2005), a global dataset containing interpolated monthly average land temperatures (°C) from 1901 onward in a grid of spatial coordinates (0.5° × 0.5°). I used the most recent temperature data (“cru_ts_3.23.2011.2014,” downloaded on September 26, 2015) comprising monthly temperature means from 2011 to 2014. First, I averaged these four years to calculate mean monthly temperatures for each spatial grid cell. Second, from the resulting data file, I created a 12-layer shape file, where each layer contained a month’s mean temperature separately for each cell. Third, by intersecting temperature and species distribution shape files, I calculated the monthly mean temperatures for each species, separately for their wintering and breeding grounds. This resulted in 12 monthly means on the breeding ground, and 12 monthly means on the wintering ground for each species. For the wintering ground, I extracted the lowest monthly mean (thereafter, “nonbreeding minimum temperature”) as a proxy of winter harshness. “Seasonality” was calculated as the difference between the lowest and highest monthly mean temperatures on the breeding ground, and thus it reflects the extent of maximum thermal fluctuation during the course of a year on the breeding ground for each species. Note that neither nonbreeding minimum temperature nor seasonality reflects the true environmental conditions experienced by species with migration distances greater than 0 km. This is so, because long-distance migrants often do not experience the coldest periods on the wintering grounds (e.g., July in South America for white-rumped sandpiper *Calidris fuscicollis*), nor do they on the breeding grounds (e.g., January in North America for the same species). The latter is true for short-distance migrants too, due to their migratory tendencies under harsh environmental conditions. Therefore, both nonbreeding minimum temperature and seasonality were only used in models based on species that have a migration distance of less than 1000 km; the true values of nonbreeding minimum temperature and seasonality experienced by long-distance migrants could not be calculated due to lack of information on their temporal migratory patterns. In addition, because nonbreeding minimum temperature and seasonality could not be calculated for three fully resident species with extremely restricted distributions (i.e., *Anas laysanensis*, *Porzana atra*, *Vini stepheni*), sample size slightly varies between models with different explanatory variables.

PHYLOGENY

In order to implement the similarity of species due to common descent, I controlled for phylogenetic relatedness in all analyses. To do this I downloaded 100 random trees from www.birdtree.org (Jetz et al. 2012) using the Hackett backbone tree (Hackett et al. 2008), and repeated every model with each of these

Table 1. Models exploring the relationship between brain size and migration distance in birds with different migratory strategies.

Migration distance restriction	Entire species pool				Passerine species			
	<i>n</i>	<i>t</i> -Value	<i>P</i> -value	<i>R</i> ²	<i>n</i>	<i>t</i> -Value	<i>P</i> -value	<i>R</i> ²
No restriction	1466	-5.37	<0.0001	0.89	610	-6.44	<0.0001	0.90
>0 km	529	-3.11	0.0022	0.90	189	-4.22	<0.0001	0.92
>500 km	387	-3.60	0.0004	0.90	143	-4.49	<0.0001	0.92
>1000 km	326	-2.74	0.0067	0.92	119	-3.82	0.0002	0.93
>2000 km	233	-3.60	0.0004	0.92	78	-3.42	0.0010	0.92
>0 & <500 km	142	2.21	0.0319	0.92	46	2.47	0.0179	0.95
>500 & <1000 km	61	0.78	0.4415	0.87	24	0.48	0.6434	0.93
>2000 km & tropical wintering	146	-3.32	0.0012	0.92	58	-3.34	0.0015	0.90

Each row represents a separate model. All models include body mass as covariate (effect of body mass not shown). The first column provides the criteria used to define the species pool for each model. *t*- and *P*-values shown here are weighted averages over 100 PGLS models with different phylogenetic trees. The sign of *t*-value indicates the direction of the association, while the value shows the strength of association. Results from analyses of the entire taxonomic range and restricted analyses of passerines are provided.

random trees to control for phylogenetic uncertainty (Rubolini et al. 2015).

STATISTICAL ANALYSES

I performed phylogenetic generalized least-squares regressions (PGLS) using the “ppls” function as implemented in the R package “caper” (Orme et al. 2013). Brain size was used as a dependent variable in double predictor models, containing body mass and one of the following covariates: migration distance, nonbreeding minimum temperature, seasonality, or nonbreeding latitude. To test for the energetic trade-off hypothesis, I built double predictor models containing migration distance and body mass as explanatory variables on subsets of species over seven different migration distance intervals (i.e., migration distance ≥ 0 , >0 , >500 , >1000 , >2000 , >0 , & <500 km; >500 and <1000 km). However, because the effect of migration distance might be confounded by the effect of climate, given that these two often covary (i.e., species with longer migration distances experience milder wintering climates), I repeated the above model using a subset of species with migration distance >2000 km, and with nonbreeding range centroids within the tropics (23.4°N–23.4°S). I expect the strongest effect of migration distance on brain size in species with the longest migratory trajectories if the energetic trade-off hypothesis is to be supported. Further, to test the behavioral flexibility hypothesis, I built double predictor models containing body mass and nonbreeding minimum temperature, seasonality, or nonbreeding latitude, as explanatory variables on subsets of species with five different migration distance intervals (i.e., migration distance = 0, <500 , <1000 , >0 , and <500 km; >500 and <1000 km). I expect the strongest effect of all three variables in fully resident species, and that the strength of these associations will decrease with the length of migration distance.

All the above analyses were repeated using passerines only, since these perching songbirds (order Passeriformes) are less variable morphologically and trace their origins to a more recent common ancestor than the nonpasserines, but are more speciose and exhibit an impressive array of cognitive abilities and migratory strategies (Sol et al. 2005). Moreover, models were repeated using nonpasserine bird orders with sufficient number of species and with considerable variance in the focal explanatory variable. These orders were the *Anseriformes* and *Charadriiformes* for the energetic trade-off hypothesis and *Piciformes*, *Strigiformes*, and *Galliformes* for the behavioral flexibility hypothesis. Taxonomic order was obtained using the “tax_name” function as implemented in the R package “taxize” (Chamberlain et al. 2014), and each of the models described above was repeated with 100 random phylogenetic trees; AICc scores extracted and AICc weights were calculated. AICc weights were then used to calculate weighted mean *t* and *P*-values across the 100 models; distributions of both *t* and *P*-values of the focal explanatory variables in these model sets were plotted and are reported in Figure S1 (Table 1), and Figure S2 (Table 2) for the entire species and passerines, respectively. Phylogenetic dependence was estimated using Pagel’s λ , set to the most appropriate value assessed by maximum likelihood in each model. Brain mass and body mass were log-transformed prior to analyses; all other variables were used untransformed.

Migratory species often accumulate large amounts of fat to support their migratory flights (Newton 2008). Such body mass fluctuations might bias the results of brain size analyses in cases where migratory species have larger body masses recorded in the dataset due to accumulated fuel reserves. In these cases, relative brain size in longer-distance migrants (with more fuel accumulated) would be estimated erroneously as smaller. To rule out this confounding effect, I first tested whether mean body mass used in

Table 2. Models exploring the relationship between brain size and nonbreeding minimum temperature, seasonality, or nonbreeding latitude in birds with different migratory strategies.

Migration distance restriction	<i>n</i>	Nonbreeding minimal temperature			Seasonality			Nonbreeding latitude		
		<i>t</i> -Value	<i>P</i> -value	<i>R</i> ²	<i>t</i> -Value	<i>P</i> -value	<i>R</i> ²	<i>t</i> -Value	<i>P</i> -value	<i>R</i> ²
Entire species pool										
0 km	934/937	−2.55	0.0134	0.89	0.53	0.6029	0.89	0.34	0.7274	0.89
<500 km	1076/1079	−3.50	0.0007	0.89	1.38	0.1794	0.89	1.27	0.2153	0.89
<1000 km	1137/1140	−3.57	0.0005	0.89	1.24	0.2235	0.89	1.45	0.1572	0.89
>0 & <500 km	142	−4.14	0.0001	0.93	2.84	0.0060	0.93	3.84	0.0002	0.93
>500 & <1000 km	61	−1.83	0.0725	0.88	1.99	0.0524	0.88	2.17	0.0344	0.88
Passerines										
0 km	421	−2.77	0.0062	0.90	0.84	0.4021	0.90	1.79	0.0752	0.90
<500 km	467	−3.70	0.0003	0.90	1.43	0.1556	0.90	2.42	0.0162	0.90
<1000 km	491	−3.27	0.0012	0.90	0.75	0.4598	0.89	2.10	0.0367	0.89
>0 & <500 km	46	−4.47	0.0001	0.97	3.36	0.0017	0.96	3.42	0.0014	0.96
>500 & <1000 km	24	−1.07	0.2974	0.94	0.47	0.6453	0.94	1.36	0.1871	0.94

Each row represents a separate model. All models include body mass as covariate (effect of body mass not shown). The first column provides the criteria used to define the species pool each model was based on. Where two sample sizes are given, the first refers to the minimum temperature and the seasonality models, while the second to the nonbreeding latitude model. *t*- and *P*-values shown here are weighted averages over 100 PGLS models with different phylogenetic trees. The sign of *t*-value indicates the direction of the association, while the value shows the strength of association. Results from analyses of the entire taxonomic range and restricted analyses of passerines are provided.

the brain size models is correlated with migration distance ($n = 1466$). Second, I obtained data on minimum and maximum body mass from Dunning (2008) for 1131 bird species present in the brain dataset. Minimal and maximal body masses were obtained by averaging sexes, subspecies, and populations if separate values were available, and on the basis of these data I tested whether the ratio between minimum and mean body mass, as well as the ratio between maximum and mean body mass, covaries with migration distance. All three of these models were tested in a PGLS framework, using 100 phylogenetic trees. Results reported are weighted means (by AICc weights) of *t*- and *P*-values calculated across the 100 models. Model averaging was performed in the same fashion as with brain size models, and mean body mass and the two body mass ratios were all log-transformed prior the analyses.

Graphical presentation of data was done using residual brain masses, calculated from a log-log standard linear regression between brain mass and body mass. Fitted lines and associated standard errors were obtained from the PGLS model between the residual brain mass and the focal predictor variable, and standard errors were obtained using the “predictSE.gls” function as implemented in R package “AICcmodavg” (Mazerolle 2015). *P*-values were not adjusted for multiple comparison, in order to avoid inflation of the type II error probability (Rothman 1990, 2014). All statistical analyses and graphical representations of results were carried out in R 3.2.0 (R Core Team 2015).

Results

DATASET COVERAGE

Across the dataset (Fig. 1), migration distance varied from 0 km ($n = 937$ species) to 13,063 km in the white-rumped sandpiper (*C. fuscicollis*), while nonbreeding latitude varied from 74.61° in the Ivory Gull (*Pagophila eburnea*) to 0.01° in the spot-winged antbird (*Schistocichla leucostigma*). In species with migration distance <1,000 km, nonbreeding minimum temperature ranged from −29.05°C in the rock ptarmigan (*Lagopus muta*) to 26.84°C in the northern screamer (*Chauna chavaria*), while seasonality varied from 0.42°C in the eyebrowed jungle-flycatcher (*Rhynomyias gularis*) to 44.75°C in the Asian rosy finch (*Leucosticte arctoa*).

THE ENERGY TRADE-OFF HYPOTHESIS

Brain size is strongly negatively correlated with migration distance across the entire migratory spectrum (Table 1; Fig. 2A, D). This association disappears when fully resident species (migration distance = 0 km) were excluded from analyses (Table 1). Indeed, when analyses were restricted to short distance migrants (0–1,000 km), the negative association between brain size and migration distance did not emerge (Table 1; Fig. 2B, E) indicating that short distance migrants do not fit the relative brain size–migration distance continuum when this is assessed using the

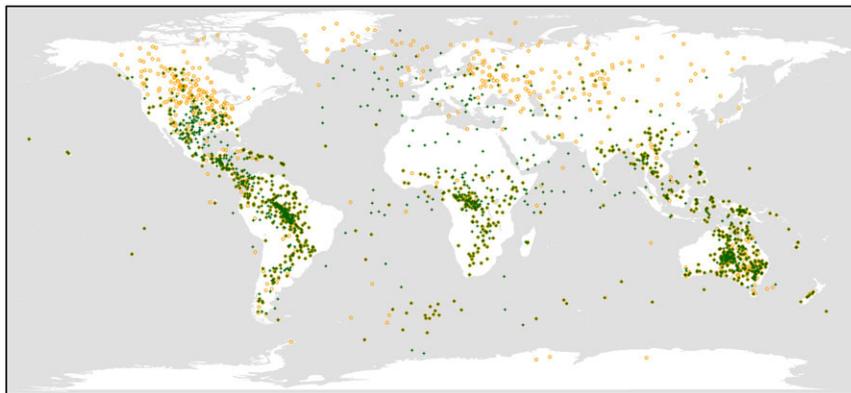


Figure 1. Map illustrating geographic data coverage. Orange circles represent the geometric centroid of the breeding areas; green pluses represent the geometric centroids of the wintering grounds of the studied species.

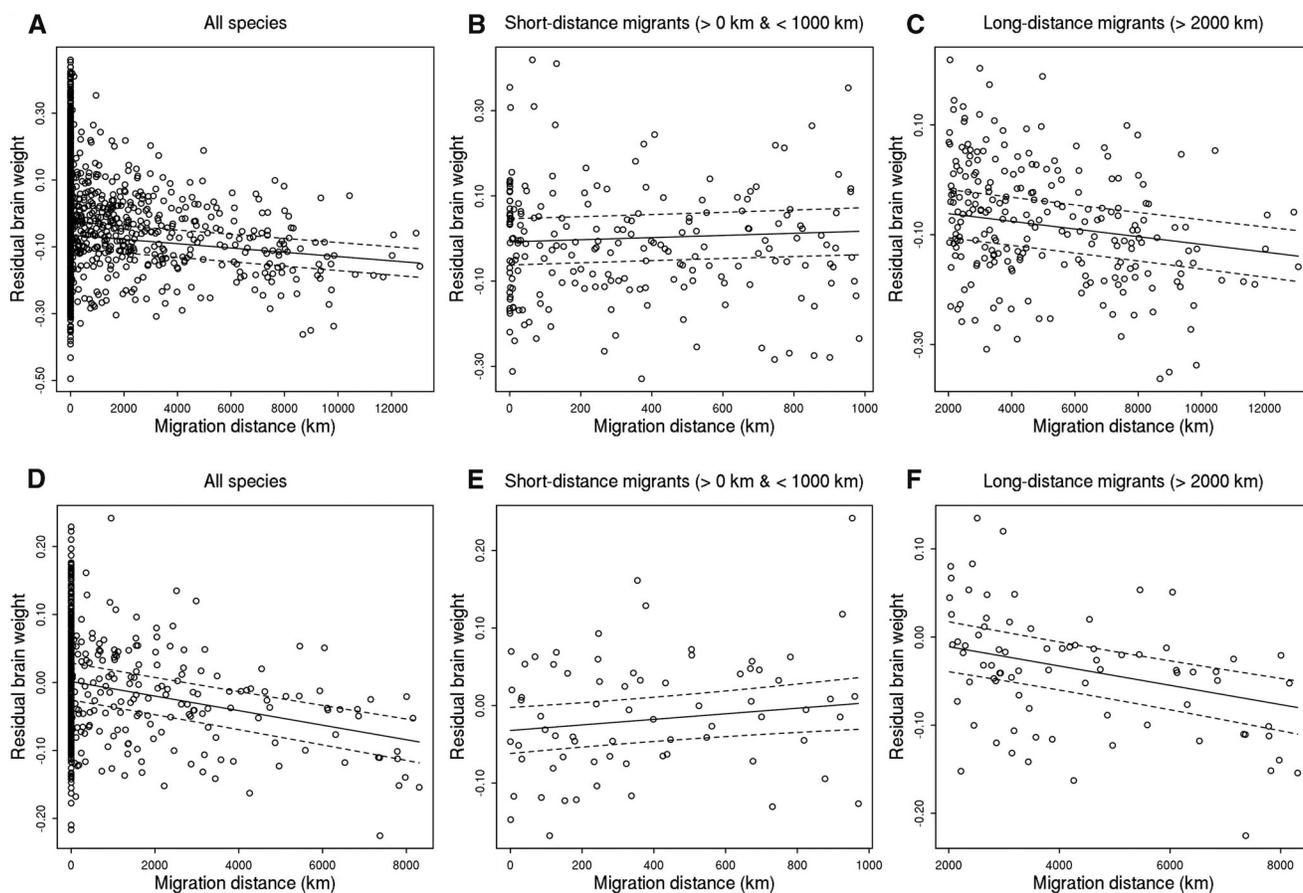


Figure 2. Association between migration distance and body-mass controlled residual brain weight in the entire species list (A), in species with migration distance between 0 and 1000 km (B), and in species with migration distance over 2000 km (C). (D–F) show the same for passerines only. Note that figures are based on raw data points, therefore much of the variation can be accounted to phylogenetic effects.

entire migratory spectrum. Nevertheless, the strength of the negative association between brain size and migratory distance increased again, despite a reduction in sample size, after short-distance migrants were excluded (i.e., subsets with migration distance from >500 to >2000 km; Table 1; Fig. 2C, F). The negative association between migration distance and brain size was also

strong for the subset of species with tropical wintering centroids and migration distances over 2000 km. All results were highly consistent when repeated just for passerines (Table 1, Fig. 2). Similar pattern was found in the case of *Charadriiformes* and *Anseriformes*, but these associations did not reach significance (Fig. S3; Table S1).

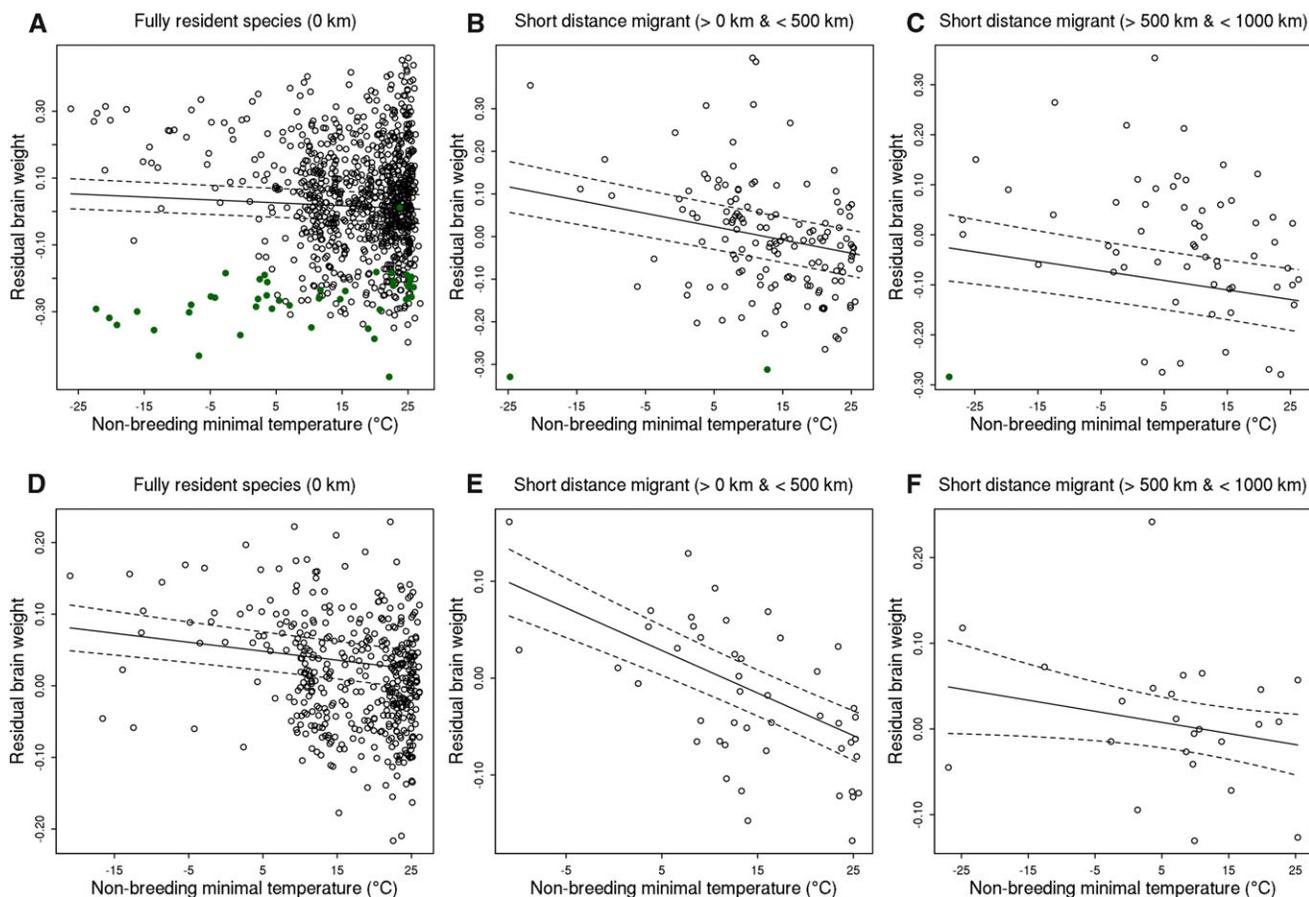


Figure 3. The association between nonbreeding minimum temperature and body-mass controlled residual brain weight in fully resident species (A), in species with migration distance between 0 and 500 km (B), and in species with migration distance between 500 and 1000 km (C). (D–F) show the same for passerines only. Note that figures are based on raw data points, therefore much of the variation can be accounted for by phylogenetic effects (e.g., green filled circles on (A–C) denote species from the *Galliformes* bird order).

Mean body mass is negatively associated with migration distance (PGLS, $n = 1466$, $t = -2.25$, $P = 0.0035$), indicating that longer-distance migratory species have lower, not higher, average body masses than species with shorter migratory distances. The ratio between minimum and mean body mass decreased slightly with migration distance (PGLS, $n = 1131$, $t = -2.54$, $P = 0.0120$), while the ratio between maximum and mean body mass was strongly positively associated with migration distance (PGLS, $n = 1131$, $t = 3.42$, $P = 0.0008$).

THE BEHAVIORAL FLEXIBILITY HYPOTHESIS

Nonbreeding minimum temperature has a strong effect on brain size in both fully resident and short-distance migratory species (Table 2, Fig. 3); the lower the nonbreeding minimum temperature, the larger the brain size (Table 2, Fig. 3). Indeed, the effect of nonbreeding minimum temperature was comparable across different migratory intervals between 0 and 500 km, but not above 500 km (Table 2). In several species subsets, nonbreeding minimum temperature is the only significant predictor of relative

brain size, while seasonality and nonbreeding latitude have little predictive power. Where significant, brain size increases with seasonality and increases with increasing nonbreeding latitude (Table 2); all results were highly consistent when repeated using just passerines (Table 2). Moreover, results were highly consistent for the *Piciformes* and the *Strigiformes* bird orders, but none of the tested environmental variables influenced brain size in the *Galliformes* bird order (Fig. S4; Table S2).

Discussion

In this study, I show in the first place that whole brain size in birds is negatively correlated with migration distance. This key result corroborates earlier studies (Sol et al. 2005, 2010; McGuire and Ratcliffe 2011), but extends this negative correlation across much wider taxonomic and geographic scales, and provides a basis for the generalization of this association outside passerines. Second, my study provides strong and clear support for the validity, and context, of the two alternative hypotheses explaining the

association between brain size and migration distance in birds, the energetic trade-off, and the behavioral flexibility hypotheses.

ENERGETIC TRADE-OFF HYPOTHESIS

Relative brain size in birds strongly decreases with increasing migration distance; this is true when considering the entire migratory spectrum, or just long-distance migrants. First, results based on the entire migratory spectrum corroborate earlier studies (Sol et al. 2005, 2010; McGuire and Ratcliffe 2011), and provide a basis for generalizing the negative association between relative brain size and migration distance across all birds. Note, however, that the negative association between brain size and migration distance was not significant in the two nonpasserine bird orders tested, both of these show similar patterns. Second, results based just on long-distance migrants provide the strongest support yet for the energetic trade-off hypothesis, indicating that it exists not just among major migration distance subdivisions (e.g., residents; short- and long-distance migrants), but also on a fine scale within just long-distance migrants. One major drawback of earlier studies is that they categorized species based on the length of their migratory trajectories; long-distance migrants were handled within just one (Sol et al. 2005; McGuire and Ratcliffe 2011), or few categories (Sol et al. 2010). Here, I provide support for the energetic trade-off hypothesis by exploring fine-scale variations of both migration distance and relative brain size within these categories, and results show that short-distance migrants do not fit on the relative brain size–migration distance linear continuum. This suggests that two different mechanisms control the evolution of the disparate relative brain sizes found in migrants and residents, and that these mechanisms act on separate ends of the migratory spectrum. In resident birds it is cognitive needs, while in migrants, energetic limitations appear to be important in regulating brain size evolution. Results suggest that shorter-distance migrants are only partially affected by both of these mechanisms.

Due to the correlative nature of this study, the negative association between migration distance and relative brain size could potentially be confounded by several factors. First, the longer the migration distance, the milder wintering conditions can get; therefore, the smaller brain size in long-distance migrants could also be explained by the year-round milder environment these birds experience. Note however that the negative association between brain size and migration distance is also apparent in species with migration distances over 2000 km, and with wintering range geometric centroids within the tropics. This result suggests that the negative association between brain size and migration distance is unlikely to be confounded by correlated climate effects, and that the nature of this association is indeed an energetic trade-off. Second, given that migratory species often accumulate large fat reserves to support their migratory flight, relative brain size might be underestimated if lean body mass is overestimated in migrants.

Such errors in the data could lead to a false identification of the energetic trade-off hypothesis as true, given that larger fuel amounts are accumulated in longer distance migrants. Note, however, that mean body mass used in the analyses was actually negatively correlated with migration distance in this dataset, indicating the migratory fuelling did not affect mean body mass estimates used here. Additionally, for a subsample of species ($n = 1131$) the ratio of minimum to mean body mass decreased slightly with increasing migration distance. This weak association compared to the strong positive association between migration distance and the ratio of maximum to mean body mass indicates that migratory fuelling is unlikely to largely distort mean body mass values used here and is therefore unlikely to confound my results.

An earlier phylogenetic path analysis showed that the largest fraction (68%) of the correlation between relative brain mass and migratory distance is a direct effect of migration on brain size (Sol et al. 2010). Although these authors argued that brain size reduction in migrants could have originated from the lowered importance of cognitive capacities in these birds (Sol et al. 2010), relative brain size in short-distance migrants is not affected by migration distance. This result is important because cognitive needs for resource exploitation in short-distance migrants might arguably be closer to those of long-distance migrants than to those of residents simply because of their migratory tendencies in case of resource shortages and their potentially decreased needs for innovation (e.g., irruptive or facultative migration; Newton 2008). Therefore, it is unlikely that the brain size of long-distance migrants shrinks simply because of a reduction in cognitive need, leaving the energetic trade-off hypothesis as a more plausible explanation. This is especially the case given that the negative association between migration distance in long-distance migratory species (over 2000 km) and with tropical nonbreeding ranges still holds true, although cognitive needs within this group of birds could potentially be similar. Second, given that migration is an extremely strenuous activity (Hedenström 2010), and the length of migration distance was shown to negatively correlate with the energetically expensive heart size (Vágási et al. 2016), I consider the pure energetic trade-off hypothesis to be the most likely explanation of brain size reduction in long-distance migrants.

THE BEHAVIORAL FLEXIBILITY HYPOTHESIS

Nonbreeding minimum temperature is a strong predictor of relative brain size in fully resident ($n = 934$ species), and short-distance (up to 500 km, $n = 142$ species) migratory birds. In other words, the colder the minimum monthly temperature on the wintering ground the larger the relative brain size of birds. Additionally, relative brain size significantly increased with nonbreeding latitude and seasonality although these effects are weaker than the association with nonbreeding minimum temperature. Thus, my

results strongly indicate that winter harshness is associated with larger brains across the avian phylogeny. Given that nonbreeding latitude and seasonality have weaker effects than nonbreeding minimum temperature on relative brain size, it is more likely that environmental severity reflected by low ambient temperature, high snow cover, and/or reduced day length (Roth and Pravosudov 2009), rather than the seasonal nature of the environment being the strongest selective force on brain size evolution in resident birds. Indeed, the importance of climate severity in brain evolution has previously been reported; food-caching black-capped chickadees from harsher wintering ranges have better spatial memory, as well as larger hippocampi and higher neuronal density in these brain regions responsible for this skill (Pravosudov and Clayton 2002; Roth and Pravosudov 2009; Roth et al. 2011). Enhanced spatial memory is thus a potential mechanism enabling birds to cope with environmental harshness, especially in food-caching species. The hippocampus occupies just a small part of total brain volume, however, and thus the results presented here must reflect additional neural adaptations to environmental severity. To date, we have very limited knowledge on how environmental conditions, in particular which aspects of the environment and in which way does it influence brain and cognitive evolution across species. The topic therefore deserves considerable future scientific attention.

FURTHER REMARKS

Change in the size of different brain regions with migration distance is nonuniform (McGuire and Ratcliffe 2011; Fuchs et al. 2014; Vincze et al. 2015), and reduction of whole brain size with increasing migration distance is mostly accounted for by reduction in the size of the telencephalon (Vincze et al. 2015), the center of higher cognitive processes. In line with the latter results, Fuchs et al. (2015) found that migratory lark sparrows (*Chondestes grammacus*) showed a clear trend toward having larger nidopalliums (a central neural substrate of higher cognitive processes in birds) than residents of the same species. Therefore, it is probable that the larger relative brain size of resident birds compared to migrants is indeed associated with their larger telencephalon and better cognitive abilities that could enhance their survival probability especially under harsh environmental conditions. It would be insightful then to consider how environmental harshness in various resident birds influences the evolution of different brain regions, on a cross-species scale. Such a follow-up study could provide more precise insights into whether increase in the size of telencephalon (and regions thereof, e.g., hippocampus) is specifically selected in species wintering under harsher environmental conditions. Additionally, whether brain size enlargement preceded, or followed, the switch in migratory habit in avian evolution is yet to be determined. Pravosudov et al. (2007) for instance examined three subspecies of white-crowned sparrow (*Zonotrichia leucophrys*) and showed that it is more likely that

brain size enlargement took place after the switch from migratory to sedentary behavior. Nonetheless, further studies should examine the nature of these associations on a broader taxonomic scale.

Relative brain size variation is subtler in bats than in birds (McGuire and Ratcliffe 2011), and the authors suggest that this discrepancy could originate from the shorter migration distances covered by bats relative to birds. In addition, I suggest that besides the longer migration distances selecting for smaller brains in both birds and mammals, harsh environments experienced by vigilant resident birds (but not hibernating resident bats) will select for enlarged brains, further distancing relative brain volume of resident from that of long-distance migratory birds.

Here I show that both environmental harshness and migration distance strongly affect brain size evolution in birds. It is important to note however that these two factors appear to explain only a fraction of the cross-species variance observed (see Figs. 2, 3). The extra variation is certainly explained by other social, ecological, physiological, or life-history factors not examined here that affect brain or cognitive evolution across birds.

Importantly, comparative studies of full brain size have been subject to strong criticism in recent years (Healy and Rowe 2007). The argument is that the brain is responsible for a wide range of functions; therefore, it is not suitable to directly associate it with specific behaviors. However, a range of studies indicate that relative brain size is a strong predictor of cognitive abilities, such as innovativity, learning, invasion, tool use, memory, and variability of habitats occupied (Sol et al. 2007, 2010; Schuck-Paim 2008; Sol 2009). Moreover, recent comparative evidence reveals that large brains in birds are a result of disproportionately enlarged pallial areas known to play key roles in avian cognition (Sayol et al. 2016). These studies suggest that whole brain size is indeed a useful tool of assessing general evolutionary patterns of brain and cognitive evolution. The results obtained this way will naturally benefit from a more specific research framework, where the change in specific brain regions is precisely assessed.

CONCLUSIONS

Here I demonstrate that increasing environmental harshness during the nonbreeding period is associated with larger relative brain sizes in both resident and short-distance migrants and thus, in these species, selection for behavioral flexibility must be an important driver of brain size evolution. Nevertheless, because I also show that increasing migration distance is linked with decreased relative brain size, the energetic trade-off hypothesis is also supported, especially in species with long migratory flights. Taken together, this study illustrates that the selection for larger brain size by cold wintering temperatures and the selection for smaller brain size by migratory flight both contribute to the evolution of disparate relative brain sizes of migratory and resident bird species, and these two mechanisms act on different ends of the

migratory spectrum. Finally, it is important to note that I have taken a correlative approach here; therefore, the nature of causalities cannot be inferred from my results. In other words, migratory habit or geographic distributions may select for larger or smaller brains, but brain size evolution might as well precede switch in migratory strategy or define suitable distribution ranges (Sol et al. 2005; Pravosudov et al. 2007).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Parameter distribution over 100 models with different phylogenetic trees presented in Table 1.

Figure S2. Parameter distribution over 100 models with different phylogenetic trees presented in Table 2.

Figure S3. Association between migration distance and body-mass controlled residual brain weight in two non-passerine bird orders, *Anseriformes* (A) and *Charadriiformes* (B).

Figure S4. Association between non-breeding minimum temperature and body-mass controlled residual brain weight in three non-passerine bird orders, *Piciformes*, *Strigiformes* and *Galliformes*.

Table S1. Models exploring the relationship between brain size and migration distance in two nonpasserine bird orders, *Anseriformes* and *Charadriiformes*.

Table S2. Models exploring the relationship between brain size and nonbreeding minimum temperature, seasonality, or nonbreeding latitude in three nonpasserine bird orders, *Piciformes*, *Strigiformes*, and *Galliformes*.

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Author for correspondence:

Orsolya Vincze

e-mail: orsolya.vincze@vocs.unideb.hu

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Evolutionary biology

Brain regions associated with visual cues are important for bird migration

Orsolya Vincze^{1,2}, Csongor I. Vágási^{1,2}, Péter L. Pap^{1,2}, Gergely Osváth^{1,2,3} and Anders Pape Møller⁴

¹MTA-DE 'Lendület' Behavioural Ecology Research Group, Department of Evolutionary Zoology and Human Biology, University of Debrecen, Egyetem tér 1, 4032, Debrecen, Hungary

²Evolutionary Ecology Group, Hungarian Department of Biology and Ecology, and ³Museum of Zoology, Babeş-Bolyai University, Clinicilor Street 5-7, 400006, Cluj-Napoca, Romania

⁴Laboratoire d'Ecologie, Systématique et Evolution, CNRS UMR 8079, Université Paris-Sud, Bâtiment 362, 91405 Orsay Cedex, France

Long-distance migratory birds have relatively smaller brains than short-distance migrants or residents. Here, we test whether reduction in brain size with migration distance can be generalized across the different brain regions suggested to play key roles in orientation during migration. Based on 152 bird species, belonging to 61 avian families from six continents, we show that the sizes of both the telencephalon and the whole brain decrease, and the relative size of the optic lobe increases, while cerebellum size does not change with increasing migration distance. Body mass, whole brain size, optic lobe size and wing aspect ratio together account for a remarkable 46% of interspecific variation in average migration distance across bird species. These results indicate that visual acuity might be a primary neural adaptation to the ecological challenge of migration.

1. Introduction

Long-distance migration in birds requires acquisition and processing of information to enable geo-positioning (map), orientation (compass) and the recognition of familiar sites [1,2]. To successfully migrate, birds use a combination of visual cues (i.e. spatial landmarks, sun, stars, colour, luminance, motion), magnetic cues and proprioceptive information [1,2]. Information processing efficiency can be achieved by an increase in the number of neurons, which would result in increased neural structure volumes, structural complexities and/or their increased neuron densities [3]. Therefore, relative enlargement of brain regions responsible for processing this information has been predicted in animals with greater need for orientation, such as migrants [1]. Here, we investigate how migration distance is associated with relative sizes of different brain regions across birds.

The regions of the avian brain that might be relevant for migration include the telencephalon, the cerebellum and the optic lobe [2–4]. Diverse information relevant for migration projects to nuclei of the telencephalon processing spatial cues (hippocampus), magnetoreception and night vision (cluster N), audition (auditory cortex), olfaction (olfactory bulb), visual cues (visual Wulst, entopallium) and putative non-compass magnetic map information (trigeminal nerve recipient hindbrain nuclei) [4–12]. The telencephalon serves various functions, and navigation and sensory information processing constitute only a fraction of these. Therefore, given the high energy demands of large brains, an overall increase in telencephalon or whole brain size can hardly be expected [13], and especially not in species with demanding life-histories, such as migrants. Indeed, the telencephalon is smaller in migratory than resident bats [14], in

Table 1. Results of multivariate PGLS models explaining variation in migration distance across 91 species of birds.

full model				minimal model			
predictor	β (s.e.)	t	p -value	predictor	β (s.e.)	t	p -value
(intercept)	-0.50 (4.58)	0.11	0.9138	(intercept)	-4.89 (2.20)	2.22	0.0290
body mass	1.02 (0.94)	1.09	0.2780	body mass	0.96 (0.80)	1.20	0.2333
aspect ratio	11.09 (2.05)	5.42	<0.0001	aspect ratio	11.05 (1.89)	5.85	<0.0001
wing area	0.32 (0.88)	0.37	0.7156	brain mass	-4.83 (1.15)	4.21	0.0001
brain mass	-15.17 (9.13)	1.66	0.1004	size of optic lobe	2.69 (1.25)	2.15	0.0345
size of telencephalon	7.29 (6.46)	1.13	0.2623				
size of optic lobe	3.69 (1.65)	2.23	0.0285				
size of cerebellum	1.44 (2.03)	0.71	0.4799				
Pagel's $\lambda = 0.76$, $n = 91$, $R^2 = 0.47$				Pagel's $\lambda = 0.80$, $n = 91$, $R^2 = 0.46$			

15 closely related songbirds [4], as well as in the migratory subspecies of dark-eyed junco (*Junco hyemalis*) [15].

The cerebellum of birds and mammals is relatively large and well developed compared with other vertebrates [16]. It coordinates skeletal muscles, and hence a well-developed cerebellum would imply fine motor dexterity, higher motion precision, and better coordination and timing during flight [17]. However, increased structural complexity and not cerebellar volume correlates with tool use and nest complexity in birds [3,18]. Additionally, the relative size of the cerebellum does not differ between sedentary and migratory bats [14]. Whether an enlarged cerebellum in birds serves as an evolutionary adaptation to long-distance flight is an open question.

The optic lobe is part of the midbrain and is well developed in birds [16,19]; it processes visual, auditory and somatosensory information. The optic tectum, the elaborately laminated supraventricular part of the lobe, is a mainly retinorecipient brain region (part of the primary visual pathway) and receives up to 90% of visual information in birds [20]. The roles of the optic tectum also include head and eye orientation towards visual and auditory stimuli, visual discrimination, spatial positioning of stimuli and motion processing [16,19,21]. Therefore, the optic lobe may play an important role in navigation, although such an association lacks evidence.

Here, we hypothesize that the size of different avian brain regions has coevolved with migration distance. We predict that longer migration distance will be associated with decreased whole brain and telencephalon size due to energetic limitations [4,22,23] and increased cerebellum and optic lobe size, because the amount of motor, visual and positional information to be processed increases with migration distance. We test these predictions using brain component sizes and migration distance for birds of six continents from a wide taxonomic range.

2. Material and methods

We extracted brain size (whole brain, telencephalon, cerebellum, optic lobe) and body mass data for 152 species of birds (electronic supplementary material, appendix S1). We calculated species-specific migration distances using distribution map shape files [24]. Wing morphology has been suggested to explain variation in migration distance [25], and, therefore, the potential

confounding effects of wing area and aspect ratio (available for 91 species) were controlled in multivariate models. We built phylogenetic generalized least squares (PGLS) models with body mass, brain region sizes, and wing morphology as explanatory variables and migration distance as the dependent variable (electronic supplementary material, appendix S1).

3. Results

Migration distance in 152 bird species ranged from 0 to 8466 km and was strongly positively correlated with wing aspect ratio across 91 species (table 1). Relative brain mass strongly decreased, while the relative size of the optic lobe increased with increasing migration distance (table 1 and figure 1). Telencephalon size decreased with migration distance in models only containing body mass and the size of telencephalon (PGLS, $n = 152$, β (s.e.) = -3.67 (0.79), $t = -4.62$, $p < 0.0001$), although this association disappeared when mass of the entire brain was included (PGLS, $n = 152$, β (s.e.) = -3.24 (3.59), $t = -0.90$, $p = 0.3685$). The size of the cerebellum was not related to migration distance (table 1; electronic supplementary material, table S1). Aspect ratio, brain mass and size of the optic lobe explained 46% of total variance in average migration distance of 91 species. These results were robust regardless of how we controlled for brain allometry, and whether we controlled for wing architecture (table 1; electronic supplementary material, table S1). Results were very similar when considering passerines only, which represent a phylogenetically, morphologically and behaviourally more uniform taxonomic group than our complete dataset (electronic supplementary material, table S1).

4. Discussion

Using data on 152 bird species from six continents and 61 families, we provide evidence of a positive association between optic lobe size and migration distance. Additionally, migration distance has a non-uniform association with different brain regions with increasing migration distance. Whole brain and telencephalon sizes decreased, while cerebellum size did not change with increasing migration distance.

The importance of high visual abilities in navigation has long been proposed [1,2]. The increase in optic lobe size

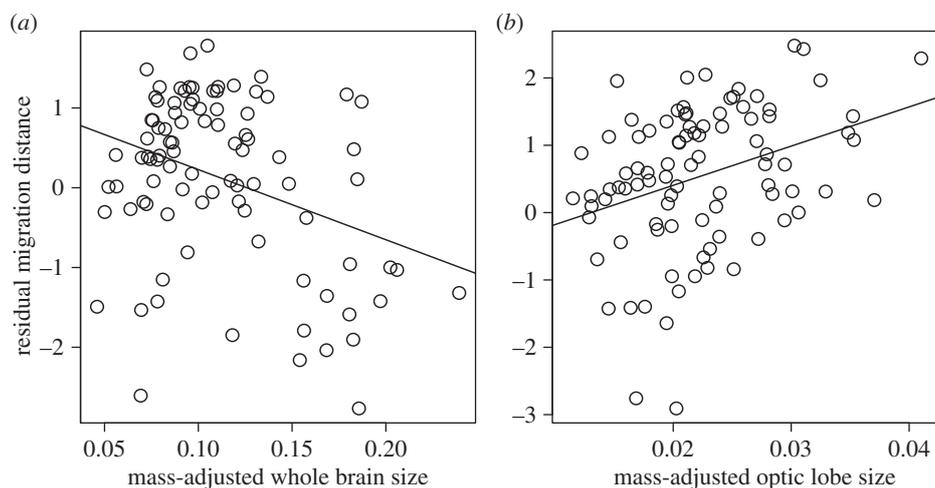


Figure 1. Residual migration distance extracted from the minimal model presented in table 1 after excluding (a) brain mass or (b) optic lobe, in relation to body mass-adjusted (a) brain mass or (b) optic lobe of different bird species. Slopes were obtained from a linear regression between the plotted variables.

with longer migration distance suggests that visual cues play a crucial role in migration. Visual cues from the environment are projected to the superficial layers of the optic tectum in the form of a topographic map (retinotopic map; [26]), while the deeper layers are motoric, guiding eye and head movement and spatial attention to salient environmental stimuli, without the need of cortical processing [19,26]. Our result indicates that the ecological challenge imposed by orientation during migration might favour the evolution of an efficient neural substrate responsible for the above capacities. Sun compass, surface reflections, motion relative to flock-mates and stabilizing visual stimuli during flight may all select for larger optic lobe in migrants, for a better visual perception and for quick flight manoeuvres. Alternatively, long-distance migrants encounter a diverse set of habitats during migration, where developed visual processing may allow for faster survey of the new environment and, therefore, better predator avoidance [27]. Note however that (i) the optic lobe is multisensory, also processing auditory and somatosensory information, which might explain the association found and (ii) apart from the tectofugal visual pathway, the thalamofugal and accessory optic pathways may also be relevant for migrants.

Both whole brain and telencephalon size decreased with migration distance, although the latter effect disappeared when whole brain size was controlled statistically. This result indicates that increasing migration distance selects for decreased whole brain size, and that the decrease in the size of telencephalon accounts for most of this overall brain size reduction. Decrease in brain size and/or telencephalon with migration has repeatedly been shown in diverse taxa [5,14,23,28]. The energy trade-off hypothesis suggests that the energetically demanding brain and migration compete for resources, which leads to a compromise in brain size. The behavioural plasticity hypothesis states that resident species experience selection for large brains because better

cognition would help them survive in seasonally changing and capricious environments [23].

Cerebellum size did not change with migration distance, suggesting that migratory flight does not depend on motor dexterity. In fact, motor dexterity has repeatedly been linked to cerebellar structure complexity rather than cerebellar volume [3,18].

Brain compartmentalization reflects the distinct selective pressures to which species are subject [3,29]. Therefore, comparative studies that link complex behaviours to brain size should handle different brain regions separately [13]. Here, we performed a detailed study of migration distance and its association with gross sections of the brain. A more powerful approach would be to study brain subdivisions on finer structural scales (e.g. hippocampus, entopallium). We further emphasize the importance of using continuous rather than categorical measures of migratory behaviours in neuroecological studies for more reliable results.

In conclusion, bird migration is associated with a smaller whole brain, smaller telencephalon and a larger optic lobe, implying that visual information might play a key role in the evolution of this behavioural syndrome.

Ethics. This study required no ethical permit, since all data were retrieved from the published literature.

Data accessibility. Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.5q034>.

Authors' contribution. A.P.M. designed the study; O.V. carried out statistical analyses; O.V., C.I.V., P.L.P. and A.P.M. wrote the manuscript with input from G.O.; all authors collected data, provided intellectual input and approved the final manuscript.

Competing interests. We declare we have no competing interests.

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1 Wing morphology and migration distance predict

2 accumulated fuel load in birds

3 Orsolya Vincze^{*a,b}, Csongor I. Vágási^{a,b}, Péter László Pap^{a,b}, Colin Palmer^c, Gareth
4 J. Dyke^a, Anders Pape Møller^d

5
6 ^aMTA-DE 'Lendület' Behavioural Ecology Research Group, Department of Evolutionary
7 Zoology, University of Debrecen, H-4032 Debrecen, Hungary;

8 ^bEvolutionary Ecology Group, Hungarian Department of Biology and Ecology, Babeş-Bolyai
9 University, RO-400006 Cluj-Napoca, Romania;

10 ^cSchool of Earth Sciences, University of Bristol, Bristol, UK;

11 ^dEcologie Systématique Evolution, Université Paris-Sud, CNRS, AgroParisTech, Université
12 Paris-Saclay, F-91405 Orsay Cedex, France

13

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16 * Corresponding author: orsolya.vincze@vocs.unideb.hu

17

18 **Summary**

19 1. Migratory birds accumulate large fat and protein reserves to fuel their long-distance flights.
20 While it is often assumed that species that migrate longest distances accumulate the largest
21 amounts of fuel, comparative evidence is scarce. Although considerable cross-species variance in
22 fuel load is seen after controlling for migration distance, it remains unclear how this variance is
23 explained by the aerodynamic attributes of different species, despite obvious ecological and
24 conservation implications.

25

26 2. We investigated maximum fuel load (MFL) in birds within the framework of a phylogenetic
27 comparative analysis. To do this, we collected data on 139 European bird species and explored four
28 questions: (1) How does MFL vary across species?; (2) Is MFL be related to migration distance
29 once phylogenetic effects are removed?; (3) Does wing morphology, as described by wing aspect
30 ratio (WAR) and wing loading (WL), influence MFL, and; (4) Does flight style influence MFL
31 across birds?

32

33 3. Our results show that MFL increases with increasing migration distance irrespective of
34 phylogenetic position. In addition, wing morphology, especially WAR has a strong effect on MFL.
35 In other words, birds with high WAR accumulate less fuel, while wing loading and flight style
36 explain little observed variation in MFL. These results highlight the complex co-evolutionary
37 relationship between migratory behavior, wing morphology, flight energetics, and fuel loading in
38 birds. We hypothesise that optimal fuel load is defined by expected energetic expenditure, the result
39 of distance and flight economy.

40

41 **Key-words** aspect ratio; body composition; fat reserves; flight range; flight style; wing loading.

42

43 **Introduction**

44 Migration is defined as the regular, active movement of animals over space. Millions of birds
45 migrate each year, flying over vast distances of up to tens of thousands of kilometers to exploit
46 seasonally variable resources (Dingle 1996; Berthold 2001, Somveille, Rodrigues & Manica 2015).
47 The longest migration distances recorded so far in birds include the approximately 11,000 km long,
48 single, non-stop trans-Pacific flight of the Bar-tailed Godwit (*Limosa lapponica baueri*) (Gill et al.
49 2009) and the more than 90,000 km travelled annually by Arctic Terns (*Sterna paradisaea*) from
50 the Arctic to the Antarctic and back (Fijn et al. 2013). Such strenuous movements obviously
51 impose strong natural selection on physiology, morphology, and the behaviour of migrants (Newton

53 2008). The intensity of selection is also illustrated by the often extremely high mortality rates
54 experienced by species during migration, higher than during any other part of annual cycles (Owen
55 & Black 1989, Guillemain et al. 2010).

56 In order to deal with the exceptionally high energetic demands of sustained, high intensity
57 migratory flight, birds accumulate fuel prior to departure and at stopover sites en route (Lindstöm
58 1991; Barlein 2003). This process is called pre-migratory fattening, as birds consume food in
59 excess (hyperphagia) and gain weight at a very fast rate, attaining maximum deposition rates of
60 between 10% and 15% of lean body mass per day (Lindstöm 1991). Fuel reserves are especially
61 important and reach maximum levels before long spells of non-stop flight, usually over ecological
62 barriers such as seas and deserts where there are no feeding opportunities (Bairlein 2003).

63 Migratory birds commonly store large reserves of fuel prior to crossing the Sahara Desert or the
64 Gulf of Mexico (e.g. Odum & Connell 1956; Berthold 2001), and some individuals even double
65 their weight within a short period of one-to-three weeks prior to departure (Odum & Connell 1956;
66 Hedenström & Ålerstam 1992; Biebach 1996; Newton 2008).

67 The fuel accumulated by birds for migratory flights is mostly lipids. Use of these compounds
68 as a core energy source has multifaceted benefits, the most important of which is weight economy.
69 Lipids provide seven-to-nine times more energy per unit mass than alternative oxidative fuel
70 sources, including proteins and carbohydrates; thus, they are both rich in energy and light to be
71 transported (McWilliams et al. 2004, Newton 2008). In addition, as fat is deposited subcutaneously,
72 it augments thermoregulation and buoyancy, imparts mechanical protection, and can even serve as
73 social or sexual signal (Mortensen & Blix 1986; Witter & Cuthill 1993; Lind et al. 1999). Birds are
74 exceptional among vertebrates in their ability to fuel high intensity, sustained muscle work with
75 fatty acids that are delivered from extra-muscular adipose tissues directly to working muscles by
76 the circulatory system (Jenni-Eiermann et al. 2002, McWilliams 2004). However, to achieve this,
77 lipids in birds need to be mobilised, transported, and oxidised at much higher rates than in non-
78 volant mammals (Jenni-Eiermann et al. 2002). This switch in metabolism was probably associated

79 with the evolution of flight, the need for reduced weight or WL, and/or the demand for continuous
80 energy production to fuel flight muscles.

81 While necessary for long-distance flight, accumulated fuel stores also have a wide variety of
82 disadvantages. In the first place, fuel accumulation itself is costly, because of altered behavioural
83 patterns and an increased risk of predation (Metcalf & Furness 1984). In addition, there are a
84 number of disadvantages to increased body mass, including higher metabolic rates, and an elevated
85 risk of injury or predation (Witter & Cuthill 1993). Fluctuations in body mass in migratory birds
86 also have strong aerodynamic implications. Firstly, fuel is deposited subcutaneously and this
87 increases projected body frontal area, which increases body drag, decreases lift-to-drag (L/D) ratio,
88 and increases the power required for flight (Pennycuick 1975; Hedenström & Ålerstam 1992, 1997;
89 Hedenström 2010). As fuel stores increase, energy consumed per unit distance travelled also
90 increases rapidly (Pennycuick 1975). Secondly, the accumulation of fuel increases WL which
91 elevates the cost of lift (Pennycuick 2008; Hedenström 2010). As a consequence, increasing fuel
92 loads have diminishing returns and serious implications for flight dynamics; these can include
93 decreases in manoeuvrability, angle of ascent (including dive angle), maximum flight velocity, and
94 flapping flight acceleration (Hedenström 1992). Importantly, power margin (i.e. the difference
95 between the maximum power produced by flight muscles and the power required for flight) also
96 decreases with increasing lean body mass for a given wing area; thus, the extent of body mass
97 fluctuation in birds is limited by fat-free body mass (Hedenström & Ålerstam 1997). As a
98 consequence, larger birds have smaller fuel loads relative to their lean body mass than smaller
99 species that are constrained by a narrower power margin (Hedenström & Ålerstam 1992, 1997).

100 Migratory birds have a range of morphological, physiological, and behavioural adaptations
101 to optimise their flight (e.g. Norberg 1990; Pennycuick 1998; Rayner et al. 1988, 1990; Dingle
102 1996; Berthold 2001; Hedenström 2008; Pap et al. 2015). Two optimisation strategies are
103 paramount, maximising either time- or energy-efficiency (Ålerstam & Lindström 1990). High WL
104 enables fast flight speed and helps minimise the time of migratory flight, while high WAR and low

105 WL ensure low energy expenditure by reducing flight power (Norberg 1990). Wing morphology is
106 strongly related to flight style; high AR and low WL are often associated with soaring, while high
107 WL is correlated with flapping flight (Ricklefs 1996). Of all styles, flapping flight is energetically
108 the most demanding, while soaring, gliding, and alternating flap-gliding require lower power output
109 (Ricklefs 1996). In their extensive analysis of morphological adaptations to migration, Vágási et al.
110 (2015) showed that WAR increases and WL decreases with increasing migration distance in birds.
111 These findings indicate that selection due to long-distance migration co-evolves with wing traits
112 that ensure energy-efficient flight, and therefore increase flight range for a given amount of stored
113 energy. Wing design and flight style determines the power required for flight, but it remains little
114 understood how these factors relate to energy storage before migration.

115 It is obvious that fuel store size must be carefully evaluated to safeguard premature energy
116 depletion during migratory flight, as well as to avoid the disadvantages associated with surplus
117 weight. Empirical data on how morphological, behavioural, and physiological factors explain
118 interspecific differences in fuel accumulation are limited, probably due to the difficulties inherent in
119 measuring fuel load, especially in such a way to be comparable across species with diverse fat
120 accumulation strategies (Krementz & Pendleton 1990). The fuelling capacity of migratory birds has
121 broad implications from both ecological and evolutionary perspectives. It is contingent upon human
122 land-use at stop-over sites, is influenced by climate change, and it has implications for population
123 age structure and demography, given that it directly affects survival, carry-over effects, and
124 reproductive performance between the winter and breeding season (Bairlein & Hüppop 2004).

125 In this study, we quantify fuel store across species using a novel, non-invasive technique
126 which we also validate using data on body condition. Applying species-specific fuel store data, we
127 investigate how fuelling strategy relates to phylogeny, migration distance, wing morphology, and
128 flight style. We hypothesise the use of larger fuel stores in species that travel longer distances
129 during migration, irrespective of phylogeny, smaller body masses, and wing architectures associated
130 with energetically more demanding flight (i.e. low WAR, high WL). In addition, we predict that

131 higher fuel loads should occur in species that use flapping flight (i.e. continuous flapping, passerine
132 type) compared to those that employ flap-gliding or soaring (Bruderer et al. 2010).

133

134 **Materials and methods**

135 Fuel factor

136 In order to quantify interspecific differences in fuel load, we extracted average and maximum body
137 mass data for adult males and females for each species from Cramp (1998). Species values of mean
138 and maximum body mass were then calculated as the arithmetic mean of the two sexes, and the fuel
139 factor was calculated as the ratio of maximum body mass to mean body mass. This resulted in a
140 measure that shows how many times larger maximum than mean body mass is for a given species
141 (Hedenström & Alerstam 1992).

142

143 Validation of fuel factor

144 Body size can vary significantly among different subspecies and across geographic regions of a
145 single species (Cramp 1998). Thus, intraspecific variation may bias our measure of fuel factor.
146 Therefore, we tested whether the size of maximum fuel stores can be reliably measured using fuel
147 factor derived from literature data as defined above. For validation we used data obtained at a bird
148 ringing camp in the Danube-Delta Biosphere Reserve, on the coast of the Black Sea, Romania
149 (44°32'N, 28°52'E). Activity at this bird ringing camp was concentrated around the peak of the
150 autumn migratory season (August 4th, 2014, to October 23rd, 2014); individuals captured were
151 almost exclusively migrants, as most do not breed in the area, and very few were re-captured weeks
152 or months after first encounter. Body mass, wing length, and fat and muscle score data were
153 extracted from the ringing database of species with fuel factor data, and with at least ten individuals
154 captured at the ringing camp (in total 11,786 captures of 45 species). Using these data, we built
155 separate linear regression models for each species using log body mass (dependent variable) and
156 log wing length (explanatory variable). Individual condition data were extracted from these models

157 as residual body mass, reflecting the net difference between actual and expected body mass based
158 on body size.

159 Utilising individual condition data, we first assessed whether this is best predicted by muscle
160 or fat load by building single-predictor linear regression models for each species separately, and
161 using condition and muscle or fat scores as explanatory variables. Second, to validate interspecific
162 fuel factor, we calculated a species-specific maximum condition index (henceforth ‘maximum
163 condition’, MC) as the 95th percentile of condition data for each species, where MC reflects the
164 high end of fuel accumulation of a given species during the autumn migratory period. We chose to
165 work with the 95th percentile, since extreme values including minima and maxima, are often
166 highly unreliable and prone to measurement or data entry errors. To analyse how fuel factor
167 correlates with MC among species, we used a weighted linear regression, where weights were
168 represented by the log number of captured individuals in the ringing database.

169

170 Estimating migration distance

171 Distribution maps of breeding and wintering ranges of western Palaearctic bird species (shape files)
172 were retrieved from <http://www.birdlife.org/datazone/info/spcdownload> (BirdLife International and
173 NatureServe 2014). Geometric centroids of spatial polygons of breeding (i.e. breeding and
174 resident) and wintering (i.e. wintering and resident) ranges were calculated from these maps using
175 the ‘gCentroid’ function of the R package ‘rgeos’ (Bivand and Rundel 2013), while migration
176 distance was calculated as the geographic distance between the breeding and wintering centroids
177 using a custom function written in R (Vágási et al. 2015, Vincze 2016). The sample of 139 species
178 analysed ranged in migration distance from 0 km (residents) to 9,360 km. Note that we only
179 gathered data on species from the western Palaearctic in order to ensure that all species had a
180 similar geographical area to cross during migration.

181

182 Wing architecture and flight style

183 We describe wing architecture as WL (kg/m²) and WAR (dimensionless). WL was calculated by
184 dividing average body mass (kg) by wing area (m²), while WAR was estimated as squared wingspan
185 divided by wing area (both m²). These parameters were either measured from pictures of stretched
186 wings using ImageJ (<http://rsbweb.nih.gov/ij/>), or from drawings of stretched wing outlines (see
187 Vágási et al. 2015 for more details). Wing parameters were measured excluding the body area
188 between the wings. Note however that wing morphology measurements including the body area or
189 not are strongly positively correlated ($R^2 > 0.99$ for both AR and wing area in 152 species, Vágási
190 et al. 2015). Because wing morphology data were not available for eight of our 139 species, sample
191 sizes vary across model sets.

192 Species were assigned to one of the four flight types listed by Pennycuick (2008) and
193 Bruderer et al. (2010), incorporating the small modifications suggested by Pap et al. (2015),
194 flapping and soaring, flapping and gliding, continuous flapping, and passerine type.

195

196 Comparative analyses

197 We conducted all analyses using phylogenetic generalised least squares (PGLS) models,
198 implemented in the R package 'nlme' (Pinheiro et al. 2015) and 'ape' (Paradis, Claude & Strimmer
199 2004). We used fuel factor as a dependent variable, with migration distance, mean body mass,
200 WAR, WL, and flight style as explanatory variables. All continuous variables were log₁₀-
201 transformed prior to analyses, and all models were weighted by the log sample size of individuals
202 measured for each species, ranging from three to 1,691. To control for similarity among taxa due to
203 common phylogenetic descent, we downloaded 1,000 random trees from birdtree.org (Jetz et al.
204 2012) using the backbone tree of Hackett et al. (2008). The rooted consensus phylogenetic tree
205 used in analyses was then obtained using the 'sumtrees' software (Sukumaran & Holder 2010), and
206 models were fitted using maximum likelihood. Phylogenetic dependence was estimated using
207 Pagel's λ , set to take the most appropriate value in each model, as evaluated by likelihood ratio
208 statistics. Model predictions and associated standard errors used for graphical presentation were

209 extracted from PGLS models using the ‘predictSE.gls’ function in the R package ‘AICcmodavg’
210 (Mazerolle 2015). We constructed models using all possible combinations of the four explanatory
211 variables, and we used second-order Akaike's Information Criterion corrected for small sample size
212 (AIC_c) to compare them and to select those with best fits. We evaluated the importance of all
213 candidate models using their relative Akaike weights (ω_i), and those of the predictors by the sums
214 of their ω_i across all models that contain the given predictor (Σ). Thus, ω_i and their sums were
215 computed for the whole model set, and all analyses were conducted in R, version 3.2.0 (R Core
216 Team 2015).

217

218 **Results**

219 Fuel factor validation

220 Results show that individual condition was strongly positively correlated with individual fat scores
221 in most species (39 out of 47, ESM 1), while the relationships between condition and muscle scores
222 were much weaker and heterogeneous. This association only reached statistical significance at the
223 5% level in 21 out of 44 species (ESM 2). Thus, these results indicate that condition reliably
224 reflects body fat content.

225 Fuel factor and maximum condition were strongly positively correlated with one another (β
226 = 0.24, SE = 0.07, $t = 3.39$, $R^2 = 0.22$, $p = 0.0011$; Figure 1); therefore, we conclude that fuel
227 factor is a suitable cross-species measure for the maximum size of stored fuels accumulated during
228 the migratory season.

229

230 Traits correlated with fuel factor

231 WAR in our sample of 131 European bird species ranged between 3.20 and 9.14 (mean = 5.04, SE
232 = 0.09), while WL ranged between 0.91 kg/m² and 11.91 kg/m² (mean = 3.01 kg/m², SE = 0.17) in .
233 Fuel factor ranged from 1.02 to 1.55 (mean = 1.23, SE = 0.01), while average body mass varied
234 between 5.6 g and 4,383 g (mean = 229.60, SE = 4.02). The fuel factor strongly increased in

235 concert with the length of migratory journey (Figure 2a, Table 1) and decreased with increasing
236 lean body mass (Figure 2b, Table 1). When wing morphology and flight style were added as
237 candidate predictor variables ($n = 131$ species), the most important predictor of fuel factor among
238 the five tested predictors was migration distance ($\Sigma = 0.98$). Thus, fuel factor strongly increased in
239 concert with migration distance travelled by a given species (Figure 2a, Table 1). WAR is also a
240 highly significant predictor of fuel factor ($\Sigma = 0.81$) as this decreases with increasing WAR (Figure
241 3a, Table 1); at the same time, WL was a less important predictor of fuel load ($\Sigma = 0.39$) as the
242 latter decreased with increasing WL, albeit a much weaker predictor (Figure 3b, Table 1). Fuel
243 factor decreased with increasing mean body mass ($\Sigma = 0.26$), although migration distance, WAR
244 and WL were all more important predictors. Flight style was a very poor predictor of accumulated
245 fuel stores ($\Sigma = 0.05$).

246

247 **Discussion**

248 Here we presented a pioneering study on cross-species variation in migratory fuelling in birds, and
249 highlights a number of key findings. First, accumulated fuel stores in birds strongly increase with the
250 geographic distance covered during migratory flight, irrespective of phylogenetic position.
251 Therefore, we hypothesize that birds adjust their fuel stores to meet total expected energy
252 expenditure of a migratory flight. Second, fuel stores are fine-tuned to fit the wing morphology of a
253 given species and therefore the energy-efficiency of their flight. Specifically, species with high WAR
254 accumulate less fuel prior to migratory departure than species with low WAR. Third, we show that
255 WL and flight style have little influence on the amount of fuel stored prior to migratory departure.

256 Migration is one of the most energy demanding behaviours seen in birds, with long distance
257 non-stop flights often assumed to be at the edge of physiological endurance (Weber 2009;
258 Hedenström 2010). Due to the energetically expensive nature of avian flight, and the frequent lack
259 of refuelling sites during migration, pre-migratory fuelling must be an important determinant of

260 flight range (Hedenström and Ålerstam 1992; Hedenström 2010). Indeed, a positive association
261 between fuel load and migration distance has been demonstrated before at the species level; the size
262 of fuel reserves in Red-billed Queleas (*Quelea quelea*) differ in accordance with the distance each
263 of the three races travel during their migration (Ward and Jones 1977). A similar pattern of fuel
264 storage was found across different Barn Swallow (*Hirundo rustica*) populations, as pre-migratory
265 fat stores increased with the length of the ecological barrier to be crossed upon departure (Rubolini
266 et al. 2002). Thus, the results presented in this study generalise these conclusions above the species
267 level; we demonstrate that the fuel stores in 139 different bird species are strongly positively
268 associated with the overall length of their migratory journeys. These finding suggest that species
269 optimise their fuel stores in proportion to the energy required for the duration of the overall
270 migratory flight.

271 WAR is the morphological character that is most strongly correlated with flight efficiency in
272 birds, where long and narrow wings, especially associated with low wing loading ensures energy
273 efficient flight (Bowlin & Wikelski 2008; Weber 2009; Vágási et al. 2015). Here we show that
274 maximal accumulated fuel stores in birds decrease with larger WAR after migration distance is
275 accounted for. This finding is in accordance with previous knowledge of flight energetics in birds;
276 the higher the energy-efficiency of the flight apparatus the lower the need for energy storage in a
277 given species (Pennycuick 2008). Our results provide the first evidence that fuel load across birds
278 evolved in line with the energetic requirements of their flight apparatus. This association suggests
279 that birds store the amount of fuel that is expected to be burnt, but no more. This claim is also
280 supported by the fact that birds often maintain stable reserve levels that are well below the
281 maximum that could possibly be obtained (Blem 1990; Witter & Cuthill 1993; Biebach 1996).
282 Weight imposed by the reserves increases the cost of flight by means of elevated wing loading and
283 associated elevated costs of locomotion (Witter & Cuthill 1993; Hedenström 2010). Large fuel
284 reserves also impose elevated metabolic expenditure, increased risk of injury or predation, impaired
285 foraging and take-off ability or impaired flight performance (Witter & Cuthill 1993; Biebach 1996).

286 The high costs of extra weight are further supported by the observation that most organs suffer
287 significant reduction in size prior to or during migration, possibly to reduce the cost of
288 transportation by reducing body weight (Battley et al. 2000). Such weight loss has to be
289 compensated by cell divisions and organ regeneration that can increase the risk of tumour
290 development and subsequent cancer (Couzin-Frankel 2015; Tomasetti and Vogelstein 2015). Facing
291 such costs indicate the high fitness benefits of a minimal body mass to be transported during
292 migration.

293 Species with large WLs for a given wing architecture may require more energy to cover a
294 given distance than species with small WLs (Schmidt-Wellenburg, Engel & Visser 2008).

295 Nonetheless, fuelling capabilities are also more limited in the former group, due to their lower
296 power margin. Our results indicate that fuel stores slightly decrease with increasing WL, but this
297 character has very little predictive power. Previous studies demonstrated no association between
298 wing loading and migration distance across species (Winkler & Leisler 2008; Vágási et al. 2015).
299 These studies illustrate that wing loading has little effect on either migratory flight or fuelling.

300 Similarly, we found no difference in fuel stores among four main flight style categories. The
301 different energetic needs of various flight styles appear to be clear and well defined (e.g., Norberg
302 1990). Nonetheless, the longest non-stop flight ever recorded for birds is associated with a
303 continuous flapping species, the bar-tailed godwit (*Limosa lapponica baueri*, Gill et al. 2009). This
304 suggests that birds might be able to economise energy expenditure irrespective of flight style.
305 Nonetheless, it is important to note that flight style is strongly related to wing morphology, as high
306 aspect ratio and low wing loading is often associated with soaring flight (Ricklefs 1996), while
307 these two wing morphology parameters might better capture fine-scale species differences in flight
308 dynamics than flight style categories.

309 In conclusion, our results indicate that pre-migratory fuel stores are optimised as a function
310 of distance to be covered and the energetics of species-specific locomotion. The optimal fuel store
311 would therefore safeguard from both starvation and the surplus costs of carried load or associated

312 physiological and environmental threats. Nonetheless, this optimisation strategy might also
313 represent a threat to migrants in a changing world. Habitat fragmentation, degradation or
314 disappearance at stop-over sites might hinder proper fuelling or re-fuelling. If no alternative fuel
315 reserves are available premature energy deficiency could lead to mass fatalities and might partly
316 explain the large scale decline in migrant bird populations.

317

318 **Authors' Contributions**

319 OV, PLP, and APM conceived the ideas and designed methodology; OV, CIV, PLP and APM
320 collected the data; OV analysed the data and led the writing of the manuscript. All authors
321 contributed critically to the drafts and gave final approval for publication.

322

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450 **Figure legends**

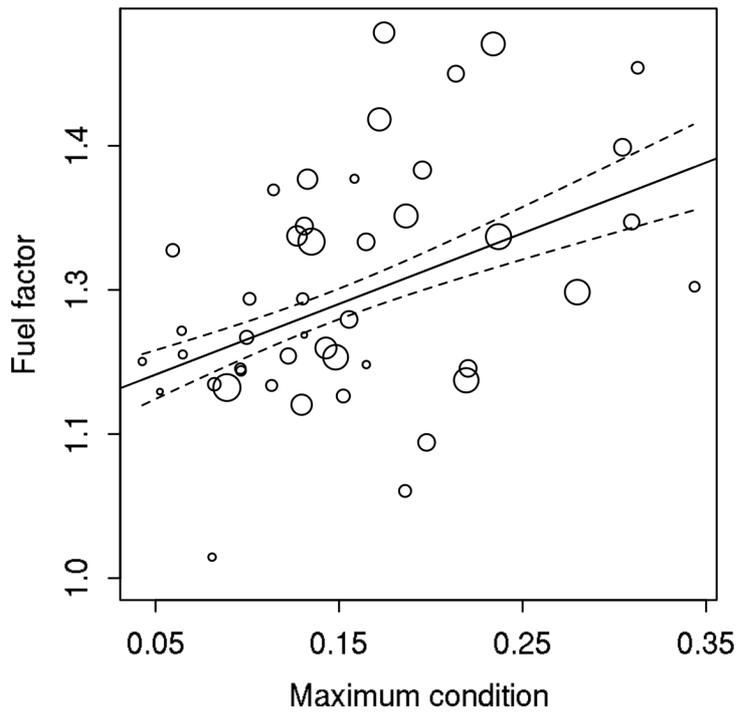
451 **Figure 1.** Association between maximum condition and fuel factor in 45 species of birds. Slope and
452 associated standard errors were obtained from a weighted linear regression between these two
453 variables. Point sizes are proportional to the log number of individuals measured for each species in
454 the bird banding camp. The lines are the linear regression line and the standard error.

455
456 **Figure 2.** Association between fuel factor and (a) migration distance (km) or (b) lean body mass
457 (g) in 139 species of birds. Slopes and associated standard errors (dashed lines) were obtained from
458 Model #1 (see Table 1). Migration distance and body mass are plotted on a log scale. Point sizes
459 are proportional to the log number of individuals for which the fuel factor estimate was based. The
460 lines are the linear regression line and the standard error.

461
462 **Figure 3.** Residual fuel factor, controlled for migration distance in 131 bird species in relation to
463 (a) wing aspect ratio (WAR) and (b) wing loading (WL). Residual fuel loads were extracted from
464 models #10 and #5, respectively (see Table 1). Slopes and associated standard errors were
465 extracted from models #5 and #6, respectively (Table 1). WAR and WL are plotted on a log scale.
466 Point sizes are proportional to the log number of individuals for which the fuel factor estimate was
467 based. The lines are the linear regression line and the standard error.

468 **Figure 1.**

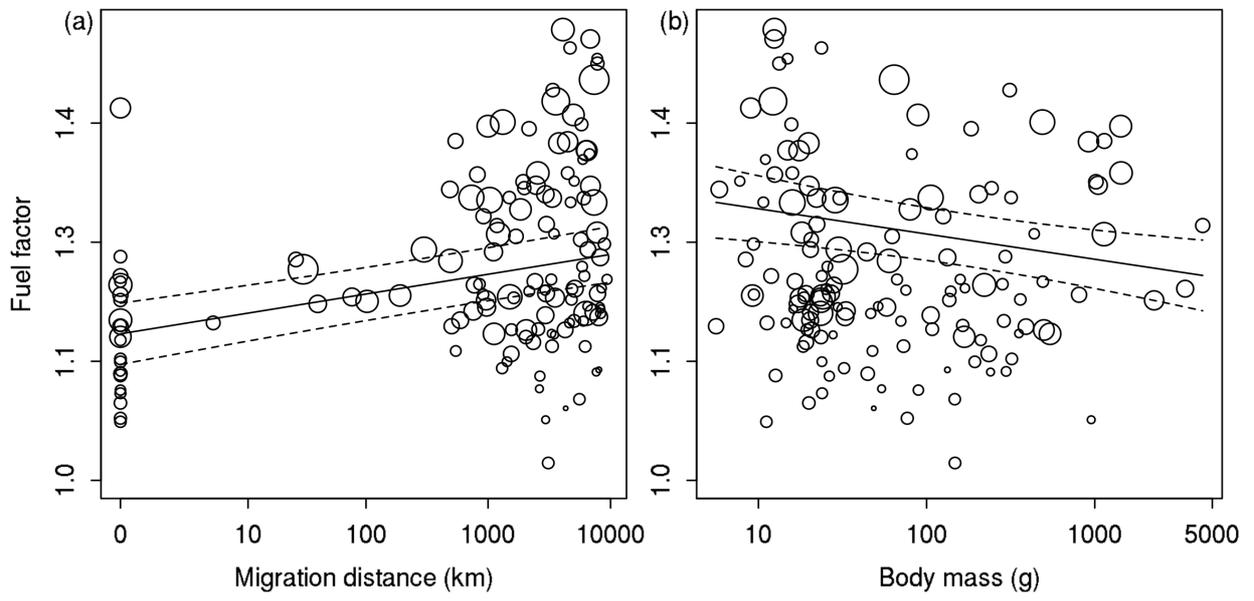
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471 **Figure 2.**

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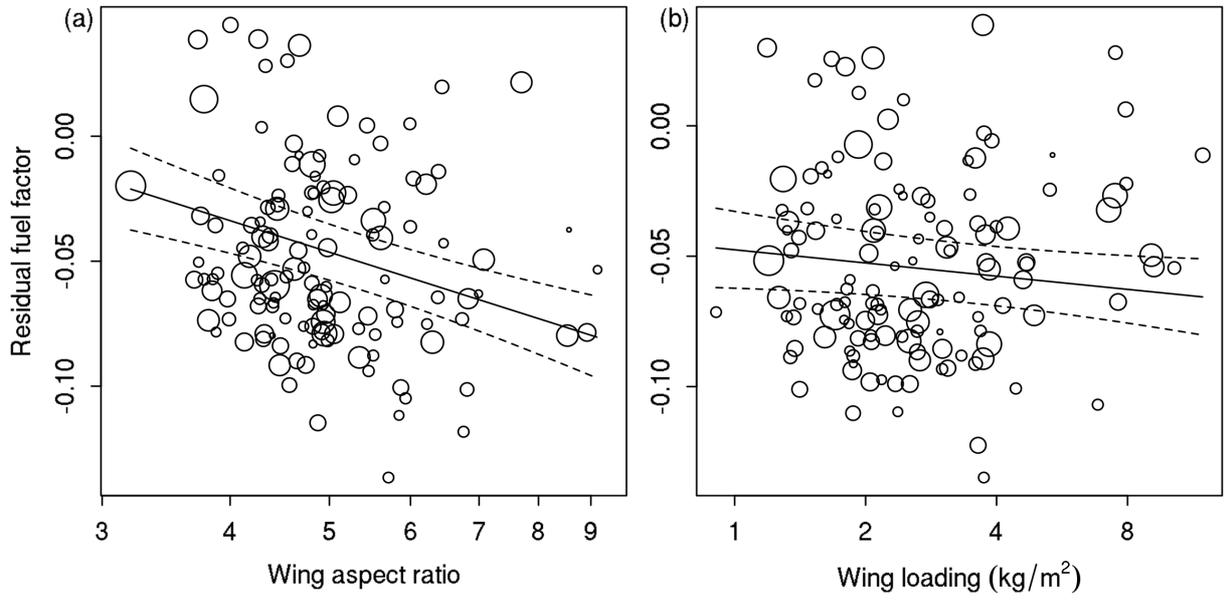
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486 **Figure 3.**

487



488 **Table 1.** Results of PGLS models explaining variation in maximum fuel load among species.
 489 Models 1 - 4 are based on 139 species, while models 5 – 36 are based on 131 species because of
 490 missing wing morphology data. Therefore, the two model sets are not directly comparable when
 491 using AICc. Abbreviations: k - number of parameters estimated, λ – phylogenetic dependence, n -
 492 sample size, AICc – second order Akaike's information criteria, Δ_{AICc} – difference from the best
 493 model's AICc, ω_i - Akaike weights, WAR – wing aspect ratio, WL – wing loading.

Model	Predictor	k	λ	n	AICc	Δ_{AICc}	ω_i
1	Migration distance + Body mass	3	0.65	139	-544.69	0.00	0.52
2	Migration distance	2	0.66	139	-544.23	0.25	0.46
3	Body mass	2	0.69	139	-536.89	7.80	0.01
4	<i>Intercept model</i>	1	0.7	139	-536.44	8.25	0.01
5	Migration distance + WAR	3	0.69	131	-521.50	0.00	0.37
6	Migration distance + WAR + WL	4	0.69	131	-520.33	1.17	0.20
7	Migration distance + WAR + Body mass	4	0.69	131	-519.44	2.06	0.13
8	Migration distance + WAR + WL + Body mass	5	0.69	131	-518.20	3.31	0.07
9	Migration distance + WL	3	0.71	131	-517.99	3.51	0.06
10	Migration distance	2	0.70	131	-517.57	3.93	0.05
11	Migration distance + Body mass	3	0.70	131	-515.97	5.53	0.02
12	Migration distance + WL + Body mass	4	0.71	131	-515.87	5.64	0.02
13	Migration distance + WAR + Flight type	6	0.68	131	-515.28	6.22	0.02
14	Migration distance + WAR + WL + Flight type	7	0.68	131	-514.23	7.28	0.01
15	Migration distance + WL + Flight type	6	0.68	131	-513.52	7.98	0.01
16	Migration distance + Flight type	5	0.68	131	-513.37	8.14	0.01
17	Migration distance + WAR + Flight type + Body mass	7	0.68	131	-513.28	8.23	0.01
18	WL	2	0.74	131	-513.01	8.50	0.01
19	Migration distance + WAR + WL + Flight type + Body mass	8	0.68	131	-511.96	9.55	0.00
20	Migration distance + Flight type + Body mass	6	0.68	131	-511.70	9.80	0.00
21	<i>Intercept model</i>	1	0.73	131	-511.57	9.94	0.00
22	Migration distance + WL + Flight type + Body mass	7	0.68	131	-511.30	10.20	0.00
23	WAR + WL	3	0.74	131	-510.95	10.55	0.00
24	WL + Body mass	3	0.73	131	-510.94	10.56	0.00
25	Body mass	2	0.73	131	-509.94	11.57	0.00
26	WAR	2	0.73	131	-509.75	11.76	0.00
27	WL + Flight type	5	0.71	131	-508.97	12.53	0.00
28	WAR + WL + Body mass	4	0.74	131	-508.86	12.64	0.00

29	WAR + Body mass	3	0.73	131	-508.00	13.51	0.00
30	Flight type	4	0.72	131	-507.56	13.94	0.00
31	WAR + WL + Flight type	6	0.71	131	-506.83	14.68	0.00
32	WL + Flight type + Body mass	6	0.71	131	-506.78	14.73	0.00
33	Flight type + Body mass	5	0.71	131	-506.03	15.48	0.00
34	WAR + Flight type	5	0.72	131	-505.40	16.10	0.00
35	WAR + WL + Flight type + Body mass	7	0.71	131	-504.59	16.91	0.00
36	WAR + Flight type + Body mass	6	0.71	131	-503.84	17.67	0.00

494