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Mitochondrial DNA density of peripheral white blood cells as a possible bio-marker of migratory status in partially migrating bird populations

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ABSTRACT

With the novelty of bird banding, radar studies, satellite tracking and systematic field observations, major advances have been made in understanding the complexity of bird migration. However, in the quest to determine the migratory status of individuals in partially migrating bird populations, most of these methods come with major disadvantages. Given that the proportion of migrants and non-migrants in partial migration populations could indicate trends in the success of the migratory process and the selection pressure on migratory bird populations, the need for research into identifying an affordable and direct method of determining the migratory status of partial migrants cannot be overemphasized. This paper generates a new platform for research into distinguishing individuals in a partially migratory populations based on acquired differences in mitochondrial biogenesis. The complexity of this kind of research and the variety of skills required for its execution, calls for the promotion of the idea.

Key words : *Partial migration, Birds, Mitochondrial energy metabolism, Biomarker.*

Introduction

Animals repeating a yearly round trip between breeding and non-breeding areas are considered to be migratory. If all the members of a population leave their breeding habitat for a migratory destination, the migration is termed 'total' as opposed to 'partial migration', in which only some members of the population migrate from the breeding area (Sinclair 1983; Terrill & Able 1988; Berthold 2001).

A partially migrating bird population may consist of mixed sub-populations of obligatory migrants and obligatory residents such that individuals exhibit a particular choice throughout life (Berthold 2001, Gill 2007). It may also consist of facultative migrants in which migration is optional for individuals leading to alternation of choices (Terrill & Able 1988; Berthold 2001; Newton 2010). Partial migration is believed to be the most widespread form of bird migration and occurs in a wide range of bird taxa including Bohemian Waxwings (*Bombycilla garrulous)*, European Robins *(Erithacus rubecula)*, Blackcaps *(Sylvia atricapilla)*, Central European Blackbird *(Turdus merula)* (Berthold 2001; Gill 2007; Newton 2010) and several species of waders such as the Black-Winged Stilt (*Himantopus himantopus*), Common Ringed Plover (*Charadrius hiaticula*), Little Ringed Plover (*Charadrius dubius*) Eurasian Oystercatcher (*Haematopus ostralegus)*. (Berthold 2001; O'brien *et al*. 2006). Some additions to the list of partially migrating waders include Purple Sandpiper (*Calidris maritime*), Black-tailed Godwit (*Limosa limosa*), Eurasian Curlew (*Numenius arquata*), Common Redshank (*Tringa totanus*), Common greens-

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hank (*Tringa nebularia*) and Common Sandpiper (*Actitis hypoleucos*) (Newton 2010). Berthold (1999) reported that many species of birds previously classified as sedentary or exclusively migratory have on more detailed studies proved to be partially migratory.

A major point of focus in avian migration research is how to distinguish migratory individuals from sedentary ones in partially migrating populations. However, existing field methods involved in establishing the migratory status of individuals in such populations are highly involving, financially demanding and tiresome due to the fact that individuals have to be tracked or recaptured several times to assess their migratory status. Thus any method with comparatively fewer disadvantages that can equally establish the migratory status of individuals in partial migrating populations would be of a major advantage for understanding the dynamics in migratory behaviour at the population level. This paper focuses on difficulties associated with current methods of assessing migratory status within partially migrating bird populations and suggests alternative direct approaches that could be developed to readily distinguish sedentary and active migrants in partially migrating bird populations.

The need for distinguishing between migratory and sedentary individuals in modern migratory bird research

Autumn migration of birds is generally thought to be triggered by limited food availability and unfavourable temperatures of energetically expensive high latitude habitats (Berthold 2001; Drent & Piersma, 2003; Piersma, 1994; Newton, 2010; Wiersma & Piersma, 1994). Although migration may have evolved to evade energetic bottlenecks, it does not come without costs. Aside the energetically tasking flight that most long distance migrant birds undergo, many individuals also stand the risks of exposure to varieties of pathogens, predators and competitors in the course of migration (Berthold 2001; Landys-Ciannelis *et al*. 2003; Newton 2007).

Recent studies on Palearctic waders revealed that competition for food resources and human disturbance peaks on tropical wintering grounds soon after autumn migrants arrive (e.g. Gbogbo *et al.* 2008). Possible consequences of these environmental conditions may include increase in the amount of energy expended in search for food, limited net gains in energy and subsequently, long term population decreases. Indeed, the populations of many migratory shorebirds such as Arctic-breeding Turnstones (*Arenaria interpres)* in South Africa and Ruffs (*Philomachus pugnax)* and Bar-tailed Godwits (*Limosa lapponica)* in Senegal, and many more species monitored in Banc d'Arguin and Ghana have experienced population declines for which no known cause has been established (Abdourahamane 2010; Underhill *et al.* 2000; Tripet & Yesou, 1998; Zwarts *et al.* 1998).

It is generally believed that partial migration evolved as a trade-off between the costs of migration and its benefits (Gill 2007). If the energetic cost of migration outweighs its benefits, sedentary lifestyle would be favoured and vice versa. Knowledge on the proportion of migratory or sedentary individuals in a partially migrating population at a particular time could thus provide an indication of the incentives versus disincentives for migratory behaviour in partially migrating populations. Once the dynamics in the proportion of migrants versus sedentary individuals in such populations are monitored over time, this information can be related to environmental factors such as limitations in food availability, climate change and habitat quality, both on breeding and non-breeding grounds. As such, major advances in understanding both the evolutionary and ecological dynamics of migratory behaviour could be achieved.

In recent times where rapid deterioration of major non-breeding habitats of many migratory bird species is the norm, it is increasingly becoming doubtful whether migration continues to offer overwhelming benefits to migrants over sedentary individuals. Evidence for this exists to the effect that the Central European Blackbird *Turdus merula* which were previously strict migrants have turned partial migrants within few decades and thus suggests that depending on the direction of selection, a partially migratory population could also become completely sedentary (Berthold, 2001, Pérez-tris & Tellería, 2002). The dynamics in the proportion of migrants and non-migrants in partially migrating populations can thus contribute to the understanding of the evolutionary and selection processes associated with bird migration.

Current methods of determining migratory status in partially migrating bird populations

At present, migrant and sedentary individuals in partially migrating bird populations are distinguished mainly by the use of body tagging (e.g. leg bands, passive integrated transponders, patagial tags), satellite tracking or the analysis of isotopes (Murray & Fuller 2000). Although these methods have been very useful to ornithologists, they have numerous disadvantages that limit their use in assessing migratory status in partially migrating populations.

The use of tags requires long-term studies and involves both intensive and extensive capture of birds at different geographic locations. It may take several years before enough recaptures or resightings can be made. Moreover, although these external body markers are quite affordable, some of them cause frictions and discomfort to birds thereby influencing mobility, survival and reproduction, which could lead to erroneous findings (Marion & Shamis 1977; Simpson & Kelsall, 1979). In addition, body tags are often lost during migration, resulting in loss of valuable information and waste of time (Andersson 1980; Murray & Fuller, 2000).

In as much as satellite tracking offers very accurate descriptions of flyways, stopovers and residing areas, both transmitters and the receiving equipment are very expensive and also have their limitations on battery life span and proper attachment (Marion & Shamis 1977; Murray & Fuller, 2000). The main problem here lies with the balancing of the transmitter size as opposed to the effect it has on the birds. Small transmitters have less negative impacts but also a shorter life span. Similar to body tags, though transmitters are secured in various ways by means of suturing, harnesses, gluing, tail mounts or implants, they are often lost, and also can influence mobility, reproduction as well as behaviour (Murray & Fuller, 2000).

The use of stable isotopes in migration studies is largely limited by the latitudinal or terrestrial to marine distribution of stable isotopes which constrains its use in short distance migrants (Hobson & Wassenaar, 1997; Kelly & Finch, 1998). Even though majority of Palaearctic migrant birds are long distance migrants, Kelly and Finch (1998) noted that isotopes have limited capacity to act as general markers that would link individual birds to specific geographic regions.

In addition to the above methods, wing moults is also used as an indicator of migratory status. The rationale is that migration and moulting are exclusive activities because the energy requirements are too large to be met simultaneously (Mead & Watmough 1976, Hall & Fransson 2001). Since many migratory birds breed in areas with short summer, wing feather moult would be severely time-constrained if it occurred on the breeding ground. Thus sedentary species typically undergo post-nuptial moult directly following breeding, whereas long-distance migrants usually do so after arrival at staging or non-breeding grounds (O'Hara *et al*. 2002). However, the difficulty in interpreting moult dynamics with respect to sedentary or migratory choices is that several other factors including timing of breeding and age tend to affect the moulting strategy (O'Hara *et al*., 2002). For instance, young individuals of many species of waders spend their boreal summer on the non-breeding ground and undergo post-nuptial moult earlier than adult migrants, which generally moult following their return (Balachandran *et al*. 2000; O'Hara *et al.* 2002). Thus in partial migrating populations, early onset of wing moult would not necessarily be an indication of sedentary life style. To overcome these difficulties, detailed knowledge of a population's moulting strategy is often required and wing moult data is often used in conjunction with other parameters including differential feather wear and body mass increments which have their own limitations (O'Hara *et al.* 2002)

As outlined above, although useful in many ways the major methods of determining migratory status are either time consuming, expensive, affect the livelihood of the birds and / or are not always accurate. Hence, the need for a more accurate, direct and affordable methods of distinguishing between migrants and sedentary individuals in partially migrating bird populations.

Prospects for alternative methods of assessing migratory status in partial migrants

Physiologically, the adoption of either a sedentary or migratory lifestyle could result in key differences among individuals, which may serve as a basis of distinguishing between migrants and non-migrants in a partially migrating population. Key biochemical differences may underlie these physiological differences. At the time active migrants need to store energy for migration, their counterpart sedentary individuals also need to store energy for breeding activities or over-wintering? However, the stored energy in migrants is expended through sustained exercise during migration, while the non-migrants are largely exempted from such prolonged exercises in the expenditure of their energy reserves (Berthold 2001). Thus differences in the mode of energy utilization may result in physiological and for that matter biochemical differences that may be exploited in determining migratory status.

Jenni-Eieamann and Jennie (1996) made earlier attempts to characterise the physiological state of birds with plasma metabolites and proposed its application in the study of migratory status (fasting and sustained exercise) of birds (Jenni-Eieamann and Jennie, 1998). Thus, differences in the levels of plasma metabolites such as triglycerides, very low density lipoproteins, glycerol, free fatty acids, and glucose, b-hydroxy-butyrate, and uric acids were proposed to characterise physiological status and could therefore be used to predict migratory status of birds.

Although the proposed method is non-invasive and can be quickly carried out, its use is constrained by factors such as capture and handling stress, together with the fact that the levels of plasma metabolites, as alluded to by the authors, are affected by the rate of disappearance and appearance and not the turnover number. To reduce the effect of handling stress in passerine birds, Jenni-Eieamann and Jennie (1998) collected blood samples as soon as birds were captured. However, trapping of many waders is carried out at night during high tides requiring that birds remain in traps until the tides recede. Thus in the use of plasma metabolites to predict the physiological conditions of waders, the effects of capture and handling stress would be more pronounced except for habitats that are suitable for the capture of birds with alternative traps such as cannon nets used during the day. But cannon nets are both financially and man-power demanding, and if not carried out with sufficient hands, can even pose more stress to the birds and thus affect plasma metabolites. Generally also, many of the proposed plasma metabolites have short half-life and levels would be altered within days and therefore constraining their use in determining migratory status. There is therefore the need for a more stable and accurate alternative physiological marker.

Alerstam *et al*. (2003) highlighted the role of physiological adaptations for fuel deposition and metabolism as one of the important determinants of migration. In relation to energy utilization, mitochondria are the cellular "powerhouse" and play major roles in cellular energy transduction (Brand 1990, Harper *et al*. 2002, Tieleman *et al*. 2008) and contribute significantly to basal, standard and field metabolic rates (Brand 1990). Thus differences in mitochondrial function and / or biogenesis between migrants and non-migrants could be explored in determining migratory status in partially migrating populations of birds.

Regular exercise leads to increased glycolysis and oxidative metabolism (Stuewe *et al*. 2000; Coleman *et al*. 1988; Murakami *et al*. 1995). Also, endurance exercise is known to be accompanied by a number of physiological adaptations that improve muscle function and exercise performance (Fitts, 2003). One of the most profound effects of endurance training is the stimulation of mitochondrial biogenesis with an increase in mitochondrial density which can be apparent after relatively few weeks of training (Holloszy & Coyle 1984; Hood, 2001). Ventura-Clapier (2009) reported that endurance exercise capacity is mainly conditioned by increased oxidative capacity, and improvement of energy fluxes and better coupling between energy production and utilization in skeletal muscle. The cytochrome c oxidase and the ATPase are important components of the mitochondrial respiratory complexes. Their activities have been strongly linked to cellular energy demand (Teodoro *et al*. 2006) and could be a key factor that distinguishes sedentary birds from migratory individuals in a partially migrating population.

In the context of finding alternative more affordable and direct methods of determining migration status, exercise increases maximal oxygen capacity ($VO_{2\ max}$). Park *et al* (1999) have shown that mtDNA density of peripheral white blood cells (pb-mtDNA) is positively correlated with $VO_{2\ max}$ in healthy humans. Thus there is the prospect that by simply drawing a small volume of blood and measuring mitochondrial DNA density, one could determine migratory status in partially migrating population of birds. Such a promising physiological marker would be particularly useful if it persists and can be used to distinguish individuals irrespective of the season of migration. If however its use is limited by 'decay' after migration, it could still be useful in collecting relevant data in short term studies. Moreover, once the rate of the decay process is known over time, the migratory status of birds can still be assessed based on such information.

In relation to the use of plasma metabolites versus mitochondrial DNA density to distinguish migrants from sedentary birds, not only do many of the proposed metabolites have short half-life and levels would be altered within days but the earlier method also requires the measurement of multiple parameters. Generally however, cell organelles would take a longer time to decay and therefore the use of mitochondrial DNA density would be a much more stable approach than plasma metabolites. In addition, mitochondria play central and direct roles in intermediary metabolism as against plasma metabolites that are affected by many other factors.

Also, mitochondrial parameters such as mitochondrial membrane phospholipid fatty acyl profile have been used in the past to determine the endurance training status (Andersson *et al*. 1998; Andersson *et al*. 2000). Even though the phospholipid fatty acyl profile is influenced by many other factors such as cold adaptation (Ocloo, *et al.*, 2007) and diet (Wander and Berdanier, 1985), and therefore likely to decay much faster than pb-mtDNA density, it has been used in determining endurance training in athletes and was found to be a promising candidate for determining ability to exercise in healthy individuals (Andersson *et al*. 1998; Andersson *et al.* 2000). The chances that a parameter such as pb-mtDNA density would quickly decay after the sustained exercise of migration would thus be low.

Despite the advantages that use of pb-mtDNA density as a physiological marker to establish migratory status would have over the existing methods, its establishment would initially involve comparison of pb-mtDNA densities in sedentary and migrants birds. These would require capturing significant numbers of birds whose migratory status and probably migratory destination would have to be studied through the same financially demanding and tiresome field methods outline earlier. However, upon a successful establishment, the migratory status of each bird that is caught in the future can be established independently of later re-captures or re-sightings and thus offering an overwhelming advantage over the existing methods.

The application of these proposed method could also be explored on differential migration in which different individuals of the same population migrate different distances. Thus pb-mtDNA densities could be studied in wader populations such as Sanderlings (*Calidris alba*) that winter in Mauritania as against their counterparts that continue to Ghana and South Africa.

Conclusion

Many of the approaches aimed at distinguishing migrants from sedentary individuals in partially migrating populations have limitations. Measurement of mitochondrial DNA density in peripheral white blood cells offers an alternative to the existing methods and could overcome some of the major limitations. There is therefore the need for studies that would focus on the differences in mitochondrial DNA density in peripheral white blood cells between active migrants and sedentary individuals in partial migratory bird populations. The successful execution of such a project will require a wide variety of skills, personnel and funds, as well as data collected for several species in various geographic locations. The promotion of this idea could however assemble such a team for the common interest of not only field ornithologist but anyone interested in partial migration.

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