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# Speciation and Metabolic rate

*Insights from an avian hybrid zone*

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### Abstract

McFarlane, S. E. 2017. Speciation and Metabolic rate. Insights from an avian hybrid zone. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1462. 43 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-554-9776-7.

The role of divergent climate adaptation in speciation has received surprisingly little scientific attention. My dissertation research focused on how resting metabolic rate (RMR) relates to the build up of prezygotic and postzygotic isolation in a natural *Ficedula* flycatcher hybrid zone. RMR is the amount of energy an organism needs to run its internal organs. Since RMR is related to life history traits and thermoregulation in other systems, it is likely to affect speciation processes at secondary contact. I found that adult collared flycatchers displace pied flycatchers into increasingly poor habitats (**Paper I**). Pied nestlings exhibit lower RMR in poor environments (**Paper II**), which may promote regional coexistence and habitat isolation by making it possible for pied flycatchers to escape competition from collared flycatchers and reduce the risk of hybridization by breeding in the poorer habitats. Further, I found that while collared flycatcher nestling RMR was not environmentally-dependent (**Paper II, Paper III**), those collared flycatcher nestlings that had a lower RMR in poor environments tended to have higher condition (**Paper III**). Further, RMR was genetically linked to a sexual ornament in collared males that has previously been shown to be beneficial in poor environments. Lastly, I found that by seven days old, nestlings increase their metabolic rate when listening to song, indicating that they are listening, and by 9 days they can discriminate between songs (**Paper IV**). Taken together, RMR could affect pre-zygotic isolation via correlations with life history strategies, song and sexual ornaments. RMR is also related to post zygotic isolation in *Ficedula* flycatchers. I found that flycatcher hybrids tended to have a higher RMR than the parental species (**Paper V**), and that there were many differentially expressed genes in energetically expensive organs in hybrids that were related to metabolic function (**Paper VI**). Thus, metabolic dysfunction, possibly caused by genetic incompatibilities, in *Ficedula* flycatcher hybrids could be a factor leading to infertility and postzygotic isolation between the parental species. Overall, I find that RMR could be a general physiological trait that affects both pre- and postzygotic isolation in hybridizing species at secondary contact, and ought to be more thoroughly considered in speciation research.

**Keywords:** resting metabolic rate, life history, hybridization, speciation, reproductive isolation, *Ficedula* flycatcher

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'A reliable interpretation of genetic data requires an understanding of ecology, evolution and behaviour'  
-Rosemary Grant

'Don't panic.'  
-Douglas Adams



# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. Rybinski J.\*, Sirkiä P.M.\*, **McFarlane S.E.\***, Vallin N., Wheatcroft D., Ålund, M., Qvarnström, A. 2016. Competition - driven build - up of habitat isolation and selection favoring modified dispersal patterns in a young avian hybrid zone. *Evolution* 70: 2226-38
- II. **McFarlane, S.E.**, Ålund, M., Sirkiä, P.M., Qvarnström, A. Adjustment of resting metabolic rate by pied flycatchers to the environment promotes regional coexistence with sister species. *Submitted manuscript*
- III. **McFarlane, S.E.\***, Ålund, M.\*, Sirkiä, P.M., Qvarnström, A. Sexual selection affects climate adaptation in collared flycatchers. *Submitted manuscript*
- IV. **McFarlane S.E.**, Sirkiä P.M., Ålund M., Qvarnström A. Hybrid Dysfunction Expressed as Elevated Metabolic Rate in Male Ficedula Flycatchers. *PLOS ONE* 11: e0161547
- V. **McFarlane S.E.**, Söderberg A., Wheatcroft D., Qvarnström A. Song discrimination by nestling collared flycatchers during early development. *Biology Letters* 12: 20160234
- VI. **McFarlane, S.E.**, Wheatcroft, D., Ålund, M., Jones, W., Xu, L., van der Valk, T., Backström, N., Ellegren, H., Qvarnström, A. *Manuscript*

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Cover picture: Original artwork courtesy of Kari Kerr.

The following papers were written during the course of my doctoral studies but are not part of the present dissertation:

- Cramer ER, Ålund M, **McFarlane SE**, Johnsen A, Qvarnström A. 2016. Females discriminate against heterospecific sperm in a natural hybrid zone. *Evolution* 70: 1844-55
- Kardos M, Husby A, **McFarlane SE**, Qvarnström A, Ellegren H. 2015. Whole - genome resequencing of extreme phenotypes in collared flycatchers highlights the difficulty of detecting quantitative trait loci in natural populations. *Molecular Ecology Resources*
- Qvarnström A, Ålund M, **McFarlane SE**, Sirkkiä PM. 2015. Climate adaptation and speciation: particular focus on reproductive barriers in Ficedula flycatchers. *Evolutionary Applications*
- Rönnegård L\*, **McFarlane SE\***, Husby A, Kawakami T, Ellegren H, Qvarnström A. 2016. Increasing the power of genome wide association studies in natural populations using repeated measures - evaluation and implementation. *Methods in Ecology and Evolution*
- Silva, C.N.S.\*, **S.E. McFarlane\***, I.J. Hagen, L. Rönnegård, A.M. Billing, T. Kvalnes, P. Kemppainen, B. Rønning, T.H. Ringsby, E.-E. Sæther, A. Qvarnström, H. Ellegren, H. Jensen and A. Husby. Insights into the genetic architecture of morphological and sexually selected traits in two passerine bird species. *in review*

\*these authors contributed equally to these manuscripts.

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# Introduction

## Secondary Contact and Reproductive Isolation

A basic principle of ecology is that species that completely compete for resources cannot coexist, since competition will result in exclusion (Hardin 1960). This problem of competition between similar species is particularly relevant when the species are related, as, in addition to competing for similar environmental resources, there is a possibility to compete for mating opportunities and hybridize, resulting in potentially less fit hybrid offspring (Coyne & Orr 2004). When closely related species come into contact after a period in allopatry (secondary contact), there are three possible outcomes. There can be competitive exclusion (Johansson & Ripa 2006), introgression and eventual merging (Seehausen et al 2008) or regional coexistence (Mittelbach & Schemske 2015). A basic tenet of speciation research seeks to understand what factors influence the likelihood of these different outcomes and which evolutionary processes can lead to maintained or increased reproductive isolation, and coexistence upon secondary contact. In addition, hybrid zones can be used to infer relationships between past evolutionary processes and the development of reproductive isolation between young species.

Reproductive isolation can be split into two categories: pre zygotic and post zygotic isolation. Prezygotic isolation refers to mechanisms that prevent different species from mating, such as habitat isolation, or behavioural isolation (Coyne & Orr 2004). Postzygotic isolation is selection against hybrid offspring, which can either be intrinsic (e.g. genetic incompatibilities), or extrinsic (e.g. hybrid offspring having intermediate phenotypes and have low fitness in both parental niches; Coyne and Orr 2004). Both these types of isolation forms can originate from evolutionary processes either in allopatry or sympatry. Further, if divergent processes have begun in allopatry, they can provide the variation between species for selection to work on while in sympatry. Species differences that have evolved in allopatry can also affect competitive interactions in sympatry that in turn effect patterns of character displacements. Thus, there can be further divergence between competing species in sympatry than there is in allopatry.

Both pre- and postzygotic isolation at secondary contact can evolve via a variety of phenotypic traits. Divergence that occurred while species were in allopatry, for example due to different climates, or to genetic drift, can provide the phenotypic variation between the species that selection acts upon in

sympatry. Subtle physiological differences between species, resulting from climate adaptation while in allopatry (Qvarnström et al 2016), can lead to prezygotic isolation by dictating different breeding seasons (Savolainen et al 2006), or post zygotc isolation, such as hybrid individuals needing intermediate thermal environments (Rasmussen et al 2012).

## Metabolic Rate

Metabolic rate is a measure of the amount of energy that an individual animal is using at any given time to power its organs and complete its life tasks (McNab 2002). Resting metabolic rate, or the amount of energy that an individual needs to run their internal organs (Burton et al 2011), has been well demonstrated to consistently scale to mass across species, where mammals tend to have a lower RMR than birds, and flying birds tends to have a higher RMR than flightless birds (McNab 2002, McNab 2012). Additionally, metabolic rate is related to thermoregulation (Naya et al 2013), is positively correlated with food availability (Mueller & Diamond 2001) and negatively correlated to temperature (Klaassen et al 2004, Song & Wang 2006) and latitude (Lovegrove 2003). The strong correlations between metabolic rate, individual animal mass, the environments in which species live, and community compositions (Sibly et al 2012), has led to the “Metabolic Theory of Ecology” which has been invoked as one of few general laws in ecology (Brown et al 2004, Humphries & McCann 2014).

Resting metabolic rate is a measure of the energetic currency that is expected to trade off between life history traits, where energy used for one trait is unavailable for other traits (Burton et al 2011, Stearns 1992). RMR could itself either positively or negatively correlate with other life history traits. A positive correlation between RMR and overall fitness is expected when a high metabolic rate is a sign of larger organs, and thus increased efficiency in energetic processing (i.e. the increased intake hypothesis; Kersten & Piersma 1987). For example, mice with large organs have high energy budgets, and thus relatively higher peak metabolic rates (Konarzewski & Diamond 1995). Conversely, a negative correlation between RMR and fitness is expected when animals downsize their internal organs so that they are less expensive and energy can be allocated to other traits (i.e. the compensation hypothesis; Deerenberg et al. 1998). For example, male storm petrels with lower RMR had offspring with faster growth rates (Blackmer et al 2005). How RMR should correlate with life history traits is likely related to the environment, where in environments with limited food resources, a negative correlation is more likely, even if efficiency is sacrificed. Divergent selection on RMR in competing species could thus lead to differences in more general life history strategies, and eventually character displacement in sympatry. Genetic correlations between RMR and life history traits could

lead to ecological character displacement, and eventual pre-zygotic isolation between closely related species. Similarly, genetic correlations between RMR and sexually selected traits could result in reproductive character displacement, and thus, prezygotic isolation. As RMR is expected to be correlated with a variety of other traits, including life history traits (Burton et al 2011), and sexually selected ornaments (Hill 2014), there is the potential for species divergence in RMR in allopatry to directly lead to prezygotic isolation and/or to facilitate pre-zygote isolation via character displacement in sympatry.

There is also a high likelihood that population divergence in RMR leads to post-zygotic isolation. RMR in hybrid individuals is expected to be disrupted when compared to the RMR of the parental species. This is because RMR is produced by the Oxidative Phosphorylation pathway (OXPHOS; Rolfe & Brown 1997), which is a cellular pathway that converts carbohydrates and oxygen to Adenosine triphosphate (i.e. molecular energy, ATP). OXPHOS is conducted across the inner mitochondrial membrane, using five compounds, which are composed of factors from both mitochondrial and nuclear genetic origin. Approximately 80% of the variation in RMR has been attributed to variation in OXPHOS (Rolfe & Brown 1997), which suggests that when OXPHOS is disrupted or inefficient, RMR is directly affected. Hybrid individuals may thus have a mitonuclear incompatibility affecting OXPHOS and thus their RMR, if their mitochondrial genome is not co-adapted to one or both copies of their nuclear genome (Burton & Barreto 2012). Hybrids with disrupted RMR have been reported in both ectothermic (Arnqvist et al 2010, Hoekstra et al 2013) and endothermic systems (Olson et al 2010, Tieleman et al 2009). Mitonuclear incompatibilities affecting the OXPHOS pathway in hybrids can lead to a) extrinsic post-zygotic isolation if a disrupted metabolic rate leads to poor performance e.g. in terms of thermoregulation of hybrids in the parental environments and eventually to b) intrinsic post-zygotic isolation, via a generally decreased energetic processing ability of hybrids (regardless of environmental conditions).

## Aims

Metabolic rate has been widely established as a general physiological mechanism in ecology (Brown et al 2004, Humphries & McCann 2014, Sibly et al 2012). However, this generally applicable and important mechanism has not, to my knowledge, been extended to research on speciation. My dissertation will attempt to extend research on resting metabolic rate to speciation questions, using a well-studied model system in speciation, *Ficedula* flycatchers.

Specifically, this dissertation addresses how resting metabolic rate relates to the speciation process in an emerging model system for speciation research. Specifically, I focus on how species divergence in the genes underlying

ing RMR affects prezygotic isolation and post zygotic isolation in a recently formed *Ficedula* flycatcher hybrid zone. The two main themes are:

1) Metabolic rate as a physiological trait affecting prezygotic isolation, specifically via the biotic environment (**Papers I, II and III**), life history traits (**Papers II and III**) and sexually selected traits (**Papers III and IV**).

2) Metabolic rate be related to post zygotic isolation, specifically affecting genetic incompatibilities (**Papers V and VI**).

# Methods

## Study Species

Collared (*Ficedula albicollis*) and pied (*Ficedula hypoleuca*) flycatchers (Figure 1) are closely related, old-world, migratory passerines. These two species recently came into secondary contact when collared flycatchers colonized Öland, a Swedish island in the Baltic in the 1960's, where pied flycatchers were already present (Qvarnström et al 2016, Qvarnström et al 2010). Collared and pied flycatchers are less than one million years diverged (Nadachowska-Brzyska et al 2013), regularly hybridize when in sympatry (Cramer et al 2016, Qvarnström et al 2010, Svedin et al 2008), and are obligate cavity breeders, so will readily breed in nestboxes (Lundberg & Alatalo 1992). We monitor pied and collared flycatchers inhabiting nest-boxes that we have placed in different woodlots across Öland. On a yearly basis, we record laying date, number of eggs, number of hatched nestlings and number of fledged nestlings for each flycatcher breeding attempt. We also ring, take blood and take morphological measurements from each breeding adult (females while incubating and males while feeding their nestlings) and from all nestlings in the nest that survive to 6 days old. This monitoring has been ongoing since 2002, and there are now more than 2000 nestboxes in more than 30 wooded areas on Öland for following the trajectory of these populations following secondary contact (Qvarnström et al 2010). Additionally, previous work in this system has resulted in excellent genomic resources for *Ficedula* flycatchers, including sequenced genomes (Ellegren et al 2012) and a 50K SNP chip (Kawakami et al 2014), that can be used since blood samples are taken from all individuals. We exploited the long term monitoring data collected between 2002 and 2014 in **Paper I**, and used the monitoring techniques to know the ages of nestlings in **Paper IV**.



Figure 1: From left to right, a collared flycatcher male, pied flycatcher male and a hybrid flycatcher male during the breeding season on Öland. Note that the broken collar on the hybrid male is a typical phenotype of male hybrids.

Regional coexistence of collared and pied flycatchers on Öland is achieved via life history strategy trade offs (Qvarnström et al 2009). Collared flycatchers trade off hardiness for competitive ability (Qvarnström et al 2005, Qvarnström et al 2009), where collared flycatchers pay a cost of breeding in poor environments that pied flycatchers do not. The superior competitive ability of collared flycatchers has led to the exclusion of pied flycatchers from the preferred habitat of both species (Vallin et al 2012a, Vallin et al 2012b). However, given the costs that collared flycatchers pay for breeding late in the season, when food resources are scarce, it seems that collared flycatchers are excluded from poor-quality habitats that pied flycatchers have escaped to (Vallin et al 2012a, Vallin et al 2012b). We explored the costs that collared flycatcher nestlings pay in poor environments in **Papers II & III**.

Collared and pied flycatchers readily form mixed pairs (Cramer et al 2016), and adult F1 hybrids make up approximately 5% of the breeding population (Svedin et al 2008). Hybrid females are completely sterile and lay empty eggs (Svedin et al 2008), and hybrid males also have extremely decreased fertility. Hybrid male sperm has been found to be malformed, and all nestlings raised by hybrid males were the result of extra pair copulation (Ålund et al 2013). Due to the low fertility of both male and female F1 hybrids, backcrosses are extremely rare or absent in this population (Kawakami et al 2014). We study these naturally occurring hybrids in **Papers V & VI (Figure 1)**.

## Environmental Variables (Papers I-III)

We measured temperature and food availability to determine how collared and pied flycatchers use metabolic rate to cope with their environment (**Papers II & III**). Temperature is often considered in studies of metabolism and climate adaptation (Klaassen et al 2004, Naya et al 2013, Song & Wang 2006), so we measured it during the nestling growth period. We accessed weather data from the Swedish meteorological and hydrological institute, SMHI (<http://opendata-download-metobs.smhi.se/explore/>). SMHI has two weather stations close to our study areas on Öland and in Kalmar. We used mean temperature estimates from the nearest weather stations from when the nestlings were 3 days old until when they were 12 days old (i.e. after the cross fostering), computed separately for each experimental nest.

*Ficedula* flycatchers primarily provide their offspring with caterpillars (Burger et al 2012, Cramp & Simmons 2006) that are nutrient-rich, high-quality food items (Arnold et al 2010, Eeva et al 2010). To do this, we estimated the abundance of caterpillars by quantifying larvae fecal pellets, i.e. frass (van Balen 1973, Visser et al 2006) for the most common 11 tree species in the study area, using fall traps (1x1 meter). We then extrapolated the frass counts associated with particular tree species to the breeding territory of each flycatcher pair to have an estimate of food availability for the birds included in the experiment. We combined the frass estimates associated with particular tree species with the relative densities and canopy coverage of the tree species within each particular flycatcher breeding territory. In this way, we were able to estimate caterpillar availability for all of our experimental nests (**Paper III**). We further extrapolated these estimates to all flycatcher nests in **Paper I**.

## Cross Fostering (Papers II & III)

Cross fostering is an efficient method for separating environment and genetic effects (Winney et al 2015). Offspring from one brood are transferred into a foster brood where they are reared. We used partial, reciprocal cross fostering, where two nestlings were transferred between each nest and raised by foster parents. We matched nests based on species, laying date and brood size ( $\pm 2$  nestlings), and average nestling mass. If the nests did not totally overlap in size, we moved the two largest nestlings from the smaller nest, and the two smallest nestlings from the bigger nest into the small nest. By matching by average nestling mass, we tried to ensure that nestling growth would not be affected by fostering (Hadfield et al 2013). If there was no overlap in nestling mass, we did not swap between those nests. Additionally, in 2014 and 2015, we used brood augmentation experiments to alter the available food for nestlings. We reciprocally crossed four nestlings from one

nest and two nestlings from the other, to make enlarged nests (+2 nestlings) and reduced nests (-2 nestlings), which still consisted of biological and foster nestlings. This reciprocal cross fostering allowed us to use variance partitioning to estimate and account for additive genetic variance and environmental variance (Riska et al 1985, Roff 1997).

## Respirometry (Papers II – V)

We used a custom respirometer to measure oxygen consumption, water vapour pressure and carbon dioxide production. We used an FMS respirometer, RM-8 multiplexer, PP-2H field pump and FlowBar-8, with individual glass chambers (8 x 30 cm) that birds were placed in (Sable Systems, Henderson, NV, USA; Figure 2). To measure RMR, we measured up to 7 birds at a time in individual chambers. Birds were taken from the field after 6pm, and returned before 5:30 am, to disrupt the parental feeding cycle as little as possible. Birds were kept in a climate cabinet set at 28°C, to ensure they were in their thermal neutral zone (Bushuev et al 2012, Lasiewski et al 1964). Air measurements were taken for each bird individually, and chambers were cycled through, where each chamber was measured once per second for five minutes over four times. Chamber and cycle assignment was done haphazardly. We used a flow rate of approximately 400 ml/min, consistent with previous studies of passerines of a similar size (Broggi et al 2007, Lewden et al 2012, Rønning et al 2007, Versteegh et al 2008). In **Paper IV**, we did not measure RMR. Instead, we measured metabolic rate before and after song treatments to determine if there was a difference in energy consumption when listening to song. For all measurements of metabolic rate we used Expedata (the manufacture's software) to extract readings from the respirometer.





Figure 2: Respirometry tubes for measuring resting metabolic rate of flycatchers on Öland. We had eight tubes, where one tube was always empty to act as baseline, to measure up to seven birds at one time.

### RNAseq (Paper VI)

We used RNAseq to look for transcriptional differences between collared, pied and hybrid flycatchers alimentary organs. We extracted RNA from 5 pied flycatchers, 5 collared flycatchers and 3 hybrid flycatchers to assay using RNAseq. RNA was poly-A enriched, reverse transcribed to cDNA and indexed by individual and organ type before being sequenced on an Illumina sequencing platform. After sequencing, we cross-checked individuals using SNPs that were fixed in the pure species (Kawakami et al 2014), to ensure that we had accurately assigned species. We aligned reads to the previously published *Ficedula* genome (Ellegren et al 2012), and examined differential expression in pairwise contrasts between each collared and pied flycatchers and hybrids. We also used Gene Ontology (GO) terms (Ashburner et al 2000, Primmer et al 2013) to look for functional correlations between differentially expressed genes.

# Results and Discussion

## Paper I: Competition-drive build-up of habitat isolation and selection favouring modified dispersal patterns in a young avian hybrid zone

Ecological speciation, where incipient species diverge due to different ecological selection pressures, is expected to be a general mode of speciation (Schluter 2009). One of the first reproductive barriers expected to arise from ecological speciation is habitat isolation (Coyne & Orr 2004), where there is a decreased likelihood of matings between incipient species as they segregate into the habitats that they are best adapted to. Habitat isolation can result from ecological character displacement at secondary contact, where competition between closely related species leads to use of more different habitats in sympatry compared to when they are geographically isolated (Pfennig & Pfennig 2009). Most studies on the role of habitat isolation in speciation have focused on young species and these studies have generally been constrained to infer evolutionary processes from observed patterns. Further, the relative importance of reinforcement, where hybrid pairings are selected against, leading to increased pre-zygotic isolation (Coyne & Orr 2004) is unclear in the context of ecological speciation.

In **Paper I**, we aimed to isolate process from pattern of habitat isolation, to ask if it is driven by competition or reinforcement. Specifically, we quantified an ongoing process of habitat isolation between collared and pied flycatchers. We also quantified how selection against hybridization in pied flycatchers may amplify the competition driven habitat isolation, if pied flycatcher males breeding in poor habitats hybridized less.

We combined estimates of habitat quality with 12 years of breeding data of each collared flycatcher, pied flycatcher and mixed pair nests. We found that collared flycatchers and mixed pairs bred in environments with a similar, relatively high habitat quality over time, while pied flycatcher bred in environments where the habitat quality decreased over time (Figure 3). This pattern was particularly driven by first-year breeding pied flycatchers failing to establish territories in the preferred high quality habitat. We further found that the rate of competition-driven habitat segregation could be increased due to overall selection favouring pied flycatchers breeding in poor quality habitats, as in poor habitats they have a lower risk of hybridization. These results show that the effects of competition driven habitat isolation can be strengthened through incidental reinforcement.

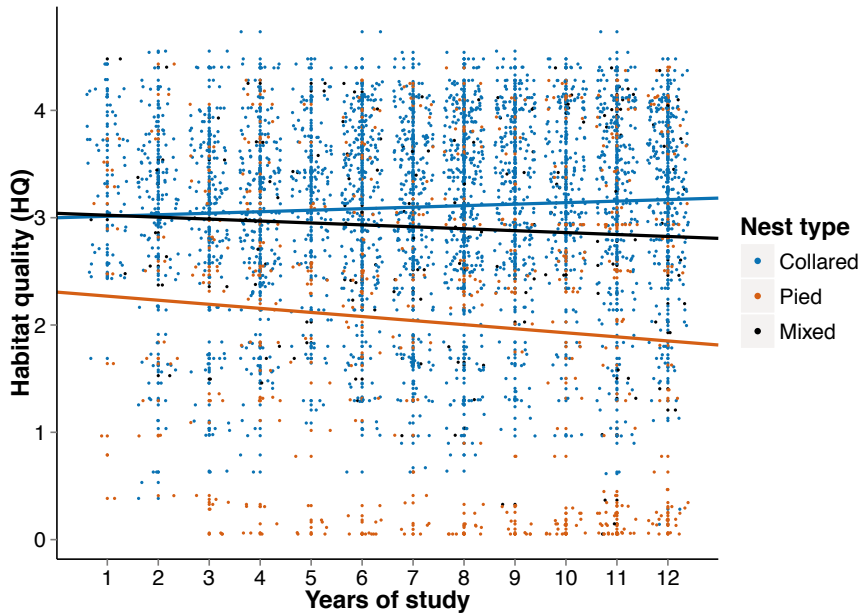


Figure 3: Over 12 years of study, we found that pied flycatcher habitat quality decreased, while the habitat quality of both collared flycatcher nests, and mixed nests stayed the same.

## Paper II: Adjustment of resting metabolic rate by pied flycatchers to the environment promotes regional coexistence with sister species

Environmental heterogeneity has been suggested to promote regional coexistence at secondary contact by leading to switches in relative fitness of species across conditions experienced (Amarasekare & Nisbet 2001, Chesson & Huntly 1997, Chesson & Warner 1981). Previous work in the flycatcher system on Öland found that pied flycatchers do not pay a cost of breeding late in the season, when there are warmer temperatures and less food, while the condition of collared flycatcher nestlings decreases as the season goes on (Qvarnström et al 2005). However, pied flycatchers cannot compete with collared flycatchers to breed in the higher quality habitat (Vallin et al 2012b). This trade off between competitive ability and hardiness in poor environments allows for regional coexistence (Qvarnström et al 2009). However, it is not clear what physiological mechanisms could promote changes in relative fitness of closely related species in different places or times. In **Paper II**, we examined whether RMR was a proximate mechanism resulting in the previously demonstrated regional coexistence of collared and pied flycatchers on Öland (Qvarnström et al 2005, Qvarnström et al 2009).

We used partial, reciprocal cross fostering to ask 1) if collared and pied flycatcher nestlings had different metabolic rates, 2) if species interacted with timing of breeding, temperature or food availability to affect RMR and 3) if RMR was heritable in both species. We found an interaction between species and timing of breeding, which appears to be driven by variation in temperature (Figure 4). Pied flycatcher nestlings have a lower RMR in warm temperatures, while collared flycatcher nestling RMR does not vary with temperature. This precisely matches the trade-off in condition between the species across the breeding season (Qvarnström et al 2005).

We found heritable variation in RMR in both species, where pied flycatcher RMR appeared to be slightly more heritable than collared flycatcher RMR. This response to temperature by pied flycatchers is most likely due to a plastic parental response to temperature, but more studies would be needed to rule out other possibilities. The differences in RMR response between the species could derive from selection pressures to different thermal environments while in allopatry, which is likely to be a common scenario at secondary contact. While it is impossible to predict the future outcomes of this system, the current interactions between RMR and temperature in pied flycatchers, but no response to temperature in collared flycatcher could be maintaining regional coexistence in this system (Qvarnström et al 2005, Qvarnström et al 2009). If collared flycatchers evolve a similar response to temperature, and pay no costs for this response, pied flycatchers could be excluded from the island. However, further character displacement could lead to pied flycatchers breeding in poorer habitats than collared flycatchers can survive in, and continued regional coexistence.

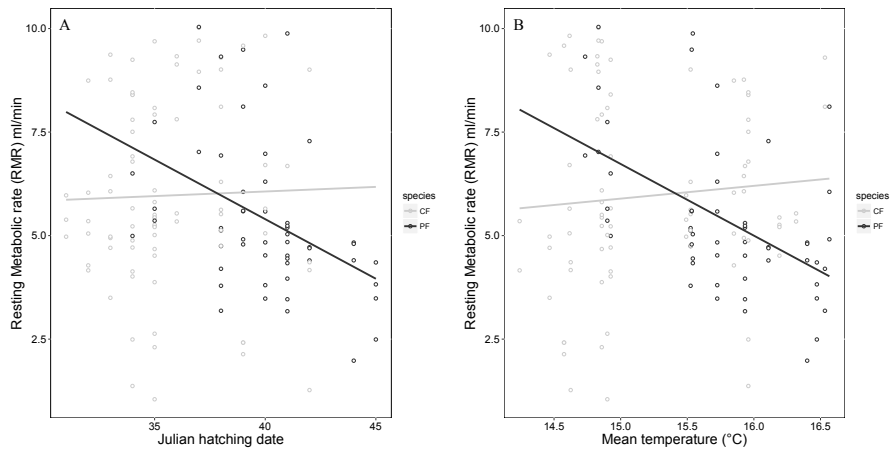


Figure 4: We found that an interaction between species and Julian hatching date explained variation in resting metabolic rate, although this interaction was no longer significant once we had accounted for multiple testing. We further explored this by testing for an interaction between species and temperature, where we found that pied, but not collared, flycatcher nestlings have different RMR depending on the temperature. This interaction was significant after adjusting for multiple testing, and explained more of the variation in RMR than hatching date did alone.

### Paper III: Sexual selection affects climate adaptation in collared flycatchers

The effect that sexual selection can have on adaptation is debated. Sexually selected traits can increase male mating success but reduce population fitness (Arnqvist & Rowe 2005, Kokko & Jennions 2014). Alternatively, good genes benefits, where sexual display traits are genetically correlated with condition, can lead to a positive correlation between sexual and natural selection, and acceleration of evolution (Lorch et al 2003, Rowe & Houle 1996). However, many good genes benefits are context dependent (Schmoll 2011), and an open question asks what physiological trait links a variable environment to sexual ornaments (Hill 2014).

In collared flycatchers, forehead patch is a well-described, condition-dependent, sexually selected trait (Gustafsson et al 1995, Qvarnström et al 2000, Robinson et al 2012, Sheldon et al 1997). Females paired with males with large patches do relatively better in poor environments (Qvarnström et al 2000) and in dry years (Robinson et al 2012). In **Paper III**, we tested whether collared flycatcher nestling RMR was a physiological trait that mechanistically linked variation in forehead patch size to context-dependent variation in fitness.

We used partial reciprocal cross fostering to disentangle genetic effects from environmental effects. First, we found a significant correlation between nestling RMR and paternal patch size, where males with larger patches had genetic nestlings with lower RMR, and that this relationship was not related to variation in temperature. Secondly, we found an interaction between nestling RMR and temperature on nestling condition, where nestlings with lower RMR had higher condition in warm temperatures (Figure 5). Taken together, this shows that nestling RMR is a consistent physiological correlate of paternal forehead patch size, which then leads to different fitness effects depending on the environment. Thus, males with relatively large forehead patches sired offspring with low metabolic rates that experienced a fitness advantage when growing under warm conditions.

If female flycatchers can use a sexual ornament to match their nestlings' RMR to the environment, then RMR is a physiological trait that accounts for the context dependent 'good genes' signalled by forehead patch size (Hill 2014). This could be a more general pattern; genetic correlations between RMR and sexual ornaments could be common across taxa, and could explain the regular occurrence of context dependent sexual selection. This could lead to implications for links between pre and post zygotic isolation, since variation in heritable sexually selected traits affects prezygotic isolation, while variation in ecological adaptation (i.e. physiology) would affect post zygotic isolation, such as if hybrid individuals are not well matched to either parental environment.

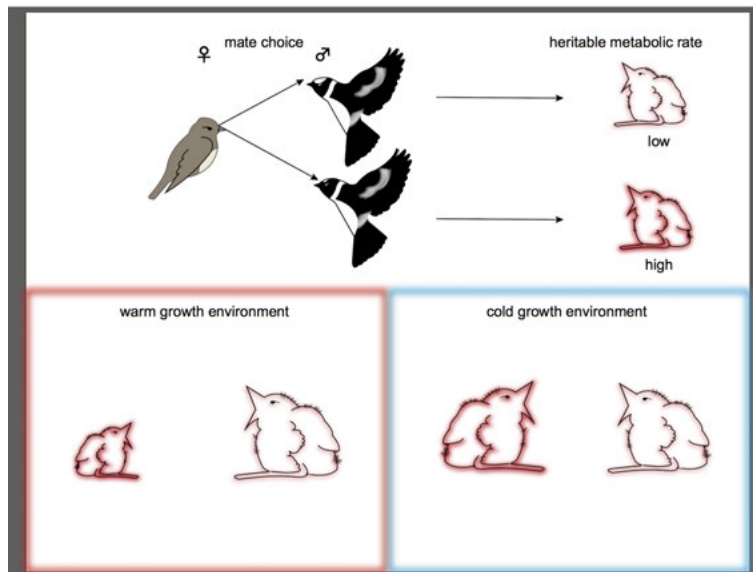


Figure 5: Female choice on male forehead patch size consistently selects for variation in metabolic rate. This variation in metabolic rate interacts with temperature to explain nestling condition, where nestlings with lower RMR have higher condition in warm environments.

## Paper IV: Song discrimination by nestling collared flycatchers during early development

Females often use male sexual signals to discriminate between conspecifics and heterospecifics when selecting their mate. When male sexually selected signals and/or female mate preferences are learned, pre-zygotic reproductive isolation can break down if heterospecific signals are learned accidentally (Verzijden et al 2012). When nestlings learn to discriminate among songs matters for song learning, because perception of auditory cues can be affected by early auditory experience (Woolley 2012). When sexual signals can be learned depends on the development of a juvenile individual; for example, auditory signals cannot be learned before the auditory pathway in the brain is complete. If auditory learning arises before auditory discrimination, there is a possibility for juveniles to learn the sexual signals of heterospecifics (Grant & Grant 1996, Woolley 2012). As collared flycatchers show behavioural discrimination when 12 days old, which is prior to the song learning period (Wheatcroft & Qvarnström submitted), we wanted to know if they can discriminate even before there are strong behavioural signals. We asked in **Paper IV**, 1) when do collared flycatcher nestlings respond to song and 2), when do collared flycatcher nestlings discriminate between songs?

We used a respirometer to measure the metabolic rate (MR) of eggs two days prior to hatching, 4-day-old nestlings, 7-day-old nestlings, 9-day-old nestlings and 12-day-old nestlings. We measured MR during a silent period, played each egg or nestling either collared flycatcher, pied flycatcher or great tit song and measured MR during the playback. We found that by 7-days-old, nestlings have a noticeably increased MR when listening to song as compared to silence, and, at 9-days-old, nestlings respond more to conspecific than to heterospecific songs (Figure 6). At 12 days we did not see this pattern, perhaps because it was masked by behavioural responses to both conspecific and heterospecific songs (Wheatcroft & Qvarnström submitted). Hence, collared flycatcher nestlings appear to respond to sound as early as when they're 7 days old, and can discriminate between songs at 9 days old. This early discrimination could contribute to strong discrimination during the song learning period, as demonstrated by the lack of mixed singing by collared flycatchers (Haavie et al 2004).



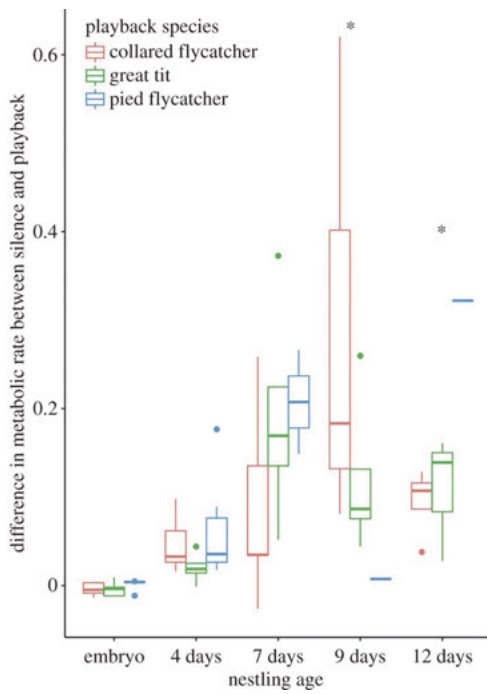


Figure 6: The differences in metabolic rate of nestlings at different ages when played either a conspecific song, pied flycatcher song, or great tit song. The asterisks note a significant interaction.

## Paper V: Hybrid Dysfunction Expressed as Elevated Metabolic Rate in Male *Ficedula* Flycatchers

The level of postzygotic isolation is thought to build up gradually during the process of speciation and is an important finishing step in the speciation process. However, the particular phenotypic traits involved are difficult to identify. Hybrid dysfunction at secondary contact can be caused by Dobzhansky Muller interactions, where alleles that have diverged in allopatry are incompatible in hybrid individuals (Dobzhansky 1936, Müller 1940). Such genetic incompatibilities might be particularly common between mitochondrial and nuclear genomes (mitonuclear), since the mitochondria mutates quickly, and could quickly fix slightly deleterious mutations which could lead to incompatibility when present in different nuclear backgrounds (Burton & Barreto 2012, Osada & Akashi 2012). As mitonuclear interactions form the basis of the OXPHOS pathway which mediates RMR (Burton & Barreto 2012, Gershoni et al 2009), and since RMR is likely to have been under divergent selection to different environments for any incipient species pairs while in allopatry (Qvarnström et al 2016), RMR and OXPHOS are likely candidates for Dobzhansky Muller interactions at secondary contact.

In **Paper V**, we measured RMR of collared, pied and F1 hybrid flycatcher males. We found that hybrid males had a significantly higher RMR than collared flycatcher males, and tended towards having a higher RMR than pied flycatcher males (Figure 7). If everything is held equal, this higher RMR is costly because of the need to allocate more energy for maintenance (Burton et al 2011). Therefore, hybrid flycatcher males appear to pay an energetic cost compared to pure species flycatchers. While asymmetries in the degree of dysfunction between hybrid types (i.e. maternal species), are common (Turelli & Moyle 2007), we did not find evidence of such an asymmetry, although our data set was limited. We conclude that, given that collared and pied flycatchers were in different refugia while in allopatry, and RMR is caused by OXPHOS, a known mitonuclear pathway, this increased RMR in hybrid flycatchers could be a symptom of a mitonuclear, Dobzhansky Muller interaction.

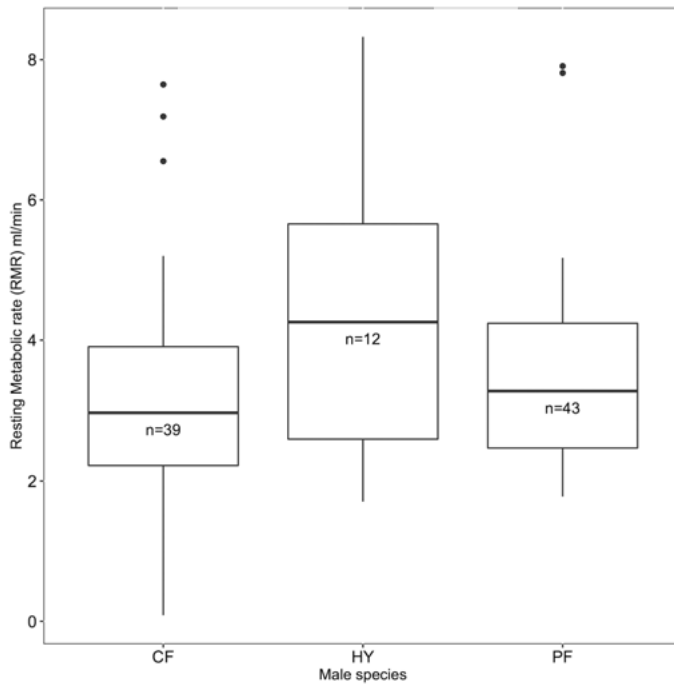


Figure 7: In a comparison between collared (CF), hybrid (HY) and pied flycatcher (PF) males, we found that hybrid flycatcher males tend to have a higher metabolic rate than either pure species.

## Paper VI: Differentially expressed genes provide insight into metabolic dysfunction in songbird hybrids

Hybrid dysfunction at secondary contact can result from epistatic interactions between genes that have diverged in allopatry, but have not yet been tested by selection when in the same individual (Dobzhansky-Muller interactions; Dobzhansky 1936, Muller 1940). While Dobzhansky-Muller interactions have been better characterized in model systems of old species pairs (Brideau et al 2006), there are fewer examples of these in relatively young species pairs (Good et al 2008). Without characterizing Dobzhansky Muller interactions in young species pairs, it is difficult to know how much they impact the speciation process, as opposed to being a by-product of other mechanisms. Recently, it has been suggested that such interactions may be even more common between mitochondrial and nuclear genomes, as these genomes are so tightly evolved (Burton & Barreto 2012, Osada & Akashi 2012). This could lead to a link between adaptation to different ecological environments and the development of genetic incompatibility. However, it is not clear if this is a general mechanism that affects hybrid incompatibilities in wild hybrid zones. *Ficedula* hybrids are an ideal system to test this hypothesis in, as there is already some evidence accumulating that there is mitonuclear conflict causing hybrid dysfunction. Specifically, *Ficedula* hybrids have decreased sperm functionality (Ålund et al 2013) and tend to have a higher RMR than the parental species (**Paper V**); both traits are affected by mitonuclear interactions (Gershoni et al 2009).

In **paper VI** we used RNAseq to evaluate 1) whether there was differential expression between the hybrids and parental species in energetically expensive alimentary organs and 2) if this differential expression was functionally related (evaluated using Gene Ontology (GO) terms; Ashburner et al. 2000, Primmer et al. 2013) to RMR and energy production. We found substantial differential expression in all pairwise contrasts, but fewer functional differences between the parental species than in the comparison between parental species and hybrids (Figure 8), suggesting dysfunction in hybrids in these gene sets. Further, we found differentially expressed genes that were part of the OXPHOS pathway, and significantly more GO terms associated with metabolic function between hybrids and the parental species than expected by chance. Thus, GO terms associated with metabolic function were over represented among the GO terms that differed between hybrids and parental species.

*Ficedula* hybrids have substantially reduced fertility (Ålund et al 2013, Svedin et al 2008), but it is not yet clear why this is the case, given how recently diverged collared and pied flycatchers are compared to most incompatible species pairs (Price & Bouvier 2002). Our findings suggest that incompatibilities in OXPHOS have evolved between collared and pied flycatchers, and could be leading to hybrid dysfunction that is characterized

phenotypically by a higher RMR than the parental species. The genomic differences between species, which do not appear to lead to large transcriptomic differences between pure species, are affecting regulation in the hybrids. This could be contributing to hybrid infertility and the lack of back-crossing between hybrids and parental species. It is possible that mitonuclear interactions affecting OXPHOS could be a general pattern across taxa causing hybrid dysfunction at secondary contact, depending on how quickly genomic differences evolve while the species are in allopatry.

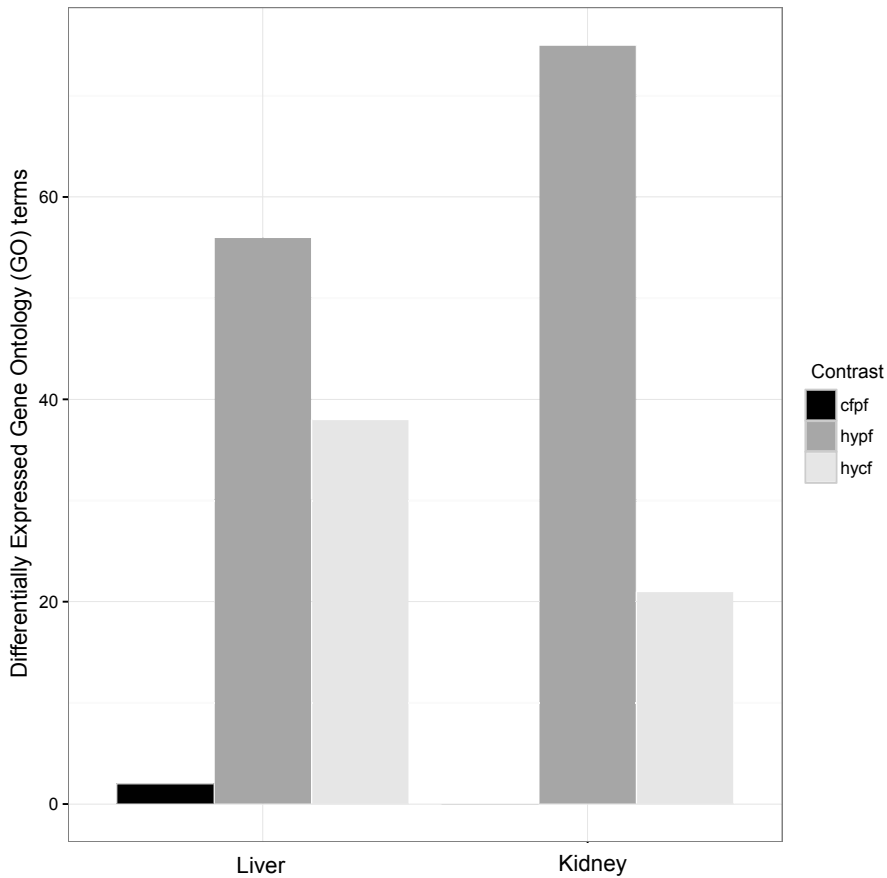


Figure 8: We found many more differentially expressed GO terms between hybrid and pied flycatchers (hypf) or hybrid and collared flycatchers (hycf) than we did in contrasts between the parental species (cfpf). This suggests that, although there are differentially expressed genes between the parental species, these genes do not necessarily result in genes in concert acting differently between parental species, while hybrids do have functional discrepancies.

## Conclusions and Future Perspectives:

In this thesis, I have considered a well-studied physiological trait, resting metabolic rate, from a new angle. I have considered how variation in RMR in two species can impact how these species coexist in a new hybrid zone, specifically looking at how RMR relates to prezygotic isolation (**Papers I, II, III and IV**) and post zygotic isolation (**Papers V and VI**). I found that RMR indeed affects the speciation dynamics in this study system. Below, I speculate on how these differences could result in changes in the interactions between the species where they coexist on Öland, and on how RMR may be important to speciation research generally.

It is possible to use the findings in this thesis to speculate about the future of the *Ficedula* hybrid zone on Öland. Given the findings in **Paper I** alone, it seems reasonable to predict that the ongoing habitat isolation and character displacement for habitat use between collared and pied flycatchers will continue and lead to stable co-existence. However, an alternative long-term outcome would be that collared flycatchers start to colonize the poor habitat. The findings in **Papers II and III** suggest that there is the potential for collared flycatchers to adapt to the poor quality habitat that pied flycatchers currently take refuge in, as we demonstrate both additive genetic variation, in **Paper II** and selection in **Paper III**. At this point in time, our results suggest that collared flycatchers cannot respond using RMR to the environment, making it difficult for them to invade also the poor quality habitat that is currently pied flycatcher refugia. However, given the variation in collared flycatcher RMR we demonstrated in **Paper III**, including some individuals with low enough RMR that they could potentially do well in poor environments. If everything else is held equal, and since RMR seems to be somewhat heritable (**Paper II**), there is thus potential for collared flycatchers to spread into poor habitats and expel pied flycatchers. However, this is a scenario that assumes no other costs or trade offs, and thus is likely overly simplistic. In the short term, it seems that pied flycatchers are likely to escape to the poor habitat.

More broadly, we have demonstrated that RMR could be related to both prezygotic isolation via competition and character displacement, and postzygotic isolation via mitonuclear conflict and Dobzhansky Muller interactions. The initial divergence in RMR between collared and pied flycatchers, which is now characterized by differences in the responsiveness of nestling RMR to the environment and mismatched regulatory functions seen in

the differential expression in hybrids, was probably the result of divergent thermal adaptation while the incipient species were in different refugia (Sætre et al 2001). As we have argued before, this is evidence that climate adaptation in allopatry can lead to both prezygotic and postzygotic isolation at secondary contact (Qvarnström et al 2016). Further, given the ubiquity of RMR and the relationship between RMR and the environment (McNab 2002, Mueller & Diamond 2001, Song & Wang 2006), suggests that RMR could regularly be a key trait involved in the speciation process.

Metabolic rate has been invoked in one of the few general ecological laws; “The Metabolic Theory of Ecology”, which states that ecological processes can be predicted by metabolic rate, and how metabolic rate scales with mass across taxa (Brown et al 2004, Humphries & McCann 2014, Sibly et al 2012). Additionally, metabolic rate has been suggested to explain variation in life history differences (Burton et al 2011) behavioural and personality differences (Biro & Stamps 2010), and other physiological differences (Chastel et al 2003, Ots et al 2001) within populations. However, such a generally applicable mechanism has not, to my knowledge, been extended to speciation research or models. I suggest that resting metabolic rate could be a general physiological trait that affects both pre- and postzygotic isolation, and that by measuring the intraspecific variation in RMR in hybridizing species at secondary contact, and understanding the consequences of RMR for competition and hybridization could lead to new, general insights for speciation research.

# Sammanfattning på svenska

När närbesläktade arter lever på samma ställe samtidigt finns det risk både för konkurrens och/eller hybridisering (det vill säga korsning mellan arterna). Sådana negativa interaktioner mellan närbesläktade arter kommer att öka eftersom klimatförändringar leder till snabba förändringar i många arters utbredning och därmed till större överlapp i deras utnyttjande av habitat. När närbesläktade arter möts, och konkurrerar eller hybridiserar, kan det leda till naturlig selektion för större skillnader mellan arterna, för att reducera konkurrensen och hybridisering. Hybridisering är nämligen ofta selekterat emot i naturen, eftersom föräldrar till hybrider ofta har lägre reproduktiv framgång jämfört med individer som parar sig inom den egna arten. Detta i sin tur kan bero på att hybrider ofta är i dåligt fysiskt skick, har svårt att föröka sig, är oattraktiva för båda arterna, är dåligt anpassade för miljön som de lever i, eller till och med är helt sterila. Evolutionära skillnader som uppkom när två arter förekom på geografiskt olika platser kan därför förstärkas via selektion som gynnar just olikheter som minskar risk för konkurrens och hybridisering när de kommer i kontakt med varandra. Utan ökade skillnader finns det risk för antingen lokal utrotning av den ena arten som ett resultat av konkurrens över resurser, eller en sammanslagning av arterna via hybridisering. Naturliga hybridzoner där närbesläktade arter överlappar och hybridiserar är därför ypperliga laboratorier både för att studera faktorer som gynnar samexistens och för att undersöka centrala processer som ligger till grund för bildandet av nya arter.

När arter förekommer geografiskt separerade från varandra kan de utveckla vissa genetiska skillnader helt av slumpen, men miljöskillnader förväntas också gynna olika lokala anpassningar. Eftersom separerade arter ofta befinner sig i olika miljöer kan vi förvänta oss att de utvecklar skillnader både i morfologiska och fysiologiska karaktärer som innebär fördelar i just de miljöer som de har anpassat sig till. På grund av detta, kan hybrider mellan två arter därför vara dåligt anpassade till miljön de föds i om de har mellanliggande morfologiska och fysiologiska anpassningar som inte matchar någon av föräldrarnas miljöer. Om den genetiska skillnaden mellan de båda föräldraarterna är stor finns det dessutom risk för problem i samarbetet mellan dessa gener i hybriderna som gör att de är sterila eller i dåligt fysiskt skick, detta eftersom interaktioner mellan föräldrarnas gener inte blivit testat av selektion när arterna var separerade. Tidigare studier av hybridzoner har, troligtvis av praktiska anledningar, fokuserat på effekter av skillnader i mor-



fologiska karaktärer mellan unga arter. Jag har istället valt att fokusera på att studera vilka effekter skillnader i en fysiologisk egenskap, vilande ämnesomsättning (VÄ), har på konkurrens och hybridisering mellan två närbesläktade sångfåglar. Den vilande ämnesomsättningen innebär den energimängd som organismen gör av med för att upprätthålla de inre organens funktion när kroppen är i vila.

Jag arbetade med halsbandsflugsnappare och svartvit flugsnappare på Öland. De utgör ett idealiskt studiesystem för att undersöka evolutionära processer i slutskedet av artbildningsprocessen eftersom de relativt nyligen blivit separata arter, antagligen för mindre än en miljon år sedan, och har endast nyligen kommit i kontakt med varandra på Öland efter att ha varit geografiskt åtskilda. Medan svartvit flugsnappare har funnits på Öland länge, har halsbandsflugsnapparen endast häckat där sedan 60-talet. Eftersom dessa fågelarter troligen fanns i olika miljöer när de var geografiskt separerade finns det potential för att de svarar olika på tids och rumsmässig variation i samma lokala miljö. Detta har även blivit fastställt i tidigare studier. Halsbandsflugsnapparen är mer konkurrenskraftig men kan inte föda upp lika många fågelungar under dåliga miljöförhållanden, medan svartvit flugsnappare inte verkar ha lika stora problem med sämre miljöer. Ungefär 5 % av häckande flugsnappare är naturligt förekommande hybrider som har en perfekt mix av svartvit- och halsbandsflugsnappares genuppsättning. Jag studerade hur VÄ interagerar med artbildning och relaterar till hybridfunktions i en flugsnappar-hybridzon på Öland.

Varför är VÄ en idealisk egenskap att mäta när man studerar konkurrens och hybridisering mellan två unga arter? Eftersom VÄ är en fysiologisk egenskap som relaterar till energikonsumtion är det en karaktär som starkt påverkar en arts konkurrenskraftighet och klimatanpassning. Generellt kan man säga att VÄ är den mängd energi som en individ behöver för att driva sina inre organ utan att svälta. All energi utöver detta som individen äter kan användas för annat, så som reproduktion och längre livslängd. Jag förväntade mig alltså att finna ett samband mellan VÄ, reproduktion, tillväxt och livslängd. Jag mätte VÄ genom att uppskatta mängden syre som fåglarna förbrukade medan de sov. I artikel II och III mätte jag VÄ hos fågelungar. Jag fann ett samband mellan VÄ och temperatur under uppväxten hos den ena arten som saknades i den andra. Svartvita flugsnapparungar hade en längre VÄ vid varmare temperaturer, alltså senare på häckningssäsongen (artikel II). Eftersom det anses vara en fördel med en sänkt VÄ vid högre yttre temperaturer och vid lägre mattillgång föreslår jag detta som en förklaring till att de svartvita flugsnapparna framgångsrikt kan häcka under dessa dåliga förhållanden, medan det går sämre för halsbandsflugsnapparna. När jag fokuserade på halsbandsflugsnapparna fann jag att VÄ är genetiskt kopplad till hanens vita pannfläck, som i tidigare studier visat sig vara attraktiv för honorna. Dessutom fann jag att fågelungar hos halsbandsflugsnappare som har lägre VÄ vid höga temperaturer tenderar att väga mer i relation till

deras storlek, vilket tyder på att det var fördelaktigt att ha en låg VÄ vid dessa temperaturer. Även om det är svårt att förutspå evolution tyder detta på att halsbandsflugsnapparungar skulle kunna utveckla en lägre VÄ för att klara av ofördelaktiga miljöer, så som svartvita flugsnappare har, för att bli bättre anpassade till de varierade miljöförhållandena på Öland.

För att utvärdera hur viktig VÄ kan vara för vuxna fåglar övervägde jag om habitatkvaliteten för de båda flugsnappararterna har förändrats över tid (artikel I). Jag mätte habitatkvalitet genom att uppskatta antalet fjärilslarver i varje habitat, eftersom båda arterna använder dem för att mata sina ungar. Jag fann att kvaliteten på halsbandsflugsnapparens häckningshabitat inte förändrats under de senaste 12 åren, men att tillgången på fjärilslarver drastiskt har minskat för den svartvita flugsnapparen. Detta beror på att halsbandsflugsnappare konkurrerar ut den svartvita flugsnapparen i de högkvalitativa habitaterna. Men jag fann också att svartvita flugsnapparhanar i lågkvalitetshabitat i medeltal producerar fler avkommor än i de bättre habitaterna. Detta beror på att risken för att av misstag reproducera med honor av fel art och producera hybridavkommor är så hög i de bättre habitaterna. Så även om dessa svartvita flugsnapparhanar måste häcka i lågkvalitativa habitat så lönar det sig eftersom de därmed inte riskerar att producera hybridavkommor. Därför kan vi komma att se flera svartvita flugsnappare som häckar i dessa sämre habitat i framtiden.

I den följande frågeställningen ville jag se ifall det fanns skillnader i VÄ mellan svartvita-, halsbands- och hybridflugsnapparhanar (artikel V). Jag fann ingen tydlig skillnad mellan svartvit- och halsbandsflugsnapparhanar, men jag fann att hybridhanar hade en högre VÄ än halsbandsflugsnapparhanar och tenderade även att ha en högre VÄ än svartvita flugsnapparhanar (artikel V). Detta antyder att hybridflugsnappare är energetiskt ineffektiva. För att följa upp det här spännande resultatet undersökte jag uttryck av gener i inre organ som behöver mycket energi för att fungera (t.e.x. lever, njurar) hos svartvita-, halsbands- och hybridflugsnappare (artikel VI). Ett avvikande uttryck av gener i dessa organ skulle kunna vara ett tecken på att hybriderna har svårt att bearbeta energi. Jag fann många gener som var uttryckt avvikande hos hybrider och fler av dessa gener var relaterade till energibearbetning eller ämnesomsättning än förväntat av slumpen. Alltså kan mina resultat tolkas som att hybridflugsnappare är energetiskt ineffektiva på grund av ett dåligt fungerande samarbete mellan generna som de har ärvt från vardera arten.

All ämnesomsättning är inte vilande ämnesomsättning. Per definition har fåglar högre ämnesomsättning än vilande ämnesomsättning när de gör något som är energetiskt krävande. Baserat på antagandet att det bör vara energetiskt krävande för fågelungar att lyssna till sång, och att lära sig känna igen den artspecifika sången använde jag mätningar av ämnesomsättning för att undersöka om och i så fall när halsbandsflugsnapparungar börjar visa respons till sång (artikel IV). Jag antog att högre ämnesomsättning när de lyss-

nade till sång betydde att fågelungarna "tänkte mer". Jag spelade sånger av halsbands- och svartvita flugsnappare och talgoxar, för ägg innan kläckning och för fågelungar som var 4, 7, 9 och 12 dagar gamla samtidigt som jag mätte deras ämnesomsättning. Jag fann att 7 dagar gamla fågelungar ökade sin ämnesomsättning när de hörde sång, och att 9 dagar gamla fågelungar ökade sin ämnesomsättning mer när de hörde sin egen arts sång. Men detta mönster fanns inte kvar vid 12 dagar gamla fågelungar, vilket kan bero på att dessa reagerar med olika beteenden för varje sångtyp, vilka alla leder till högre ämnesomsättning.

Sammanfattningsvis verkar ämnesomsättning vara en fysiologisk karaktär som är viktigt för artbildningsprocessen. Jag fann att ämnesomsättningen hos halsbands- och svartvit flugsnappare påverkar under vilka miljöförhållanden som fågelungarna kan växa upp i (artikel II & III) och hur attraktiva deras pappor är (artikel III). På grund av konkurrens med halsbandsflugsnappare, lever svartvit flugsnappare i sämre habitat än de brukade (artikel I), men förmågan till att ha lägre VÄ när det är varmare och ont om mat verkar göra det möjligt för arten att fortleva under de nya förhållandena. Därmed minskar även risken för hybridisering genom habitat isolering. Jag fann även att hybridflugsnappare tenderar att ha högre VÄ än båda föräldraarterna, och att detta kan bero på avvikande uttryck av gener i de energetiskt dyra inre organen (artikel VI). Jag har funnit rikligt med bevis för att små skillnader i lokalt anpassad ämnesomsättning mellan arter (till följt av olika utbredningsmönster) kan ha stor påverkan på interaktioner mellan arterna där deras utbredning överlappar och på artbildningsprocessen i sin helhet. Det behövs därför fler framtida studier av VÄ i hybridzoner för att undersöka om detta är en generell mekanism som påverkar artbildning.

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