Natural and Sexual Selection in a Natural Hybrid Zone of Ficedula Flycatchers

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Abstract

Speciation can be viewed as the formation of reproductive barriers between different populations. This thesis investigates patterns of natural and sexual selection shaping reproductive barriers between two hybridizing flycatchers (i.e. collared – and pied flycatchers). Behaviorally driven sexual isolation depends on both the availability of conspecific mates and on discrimination ability of individuals. My results demonstrate that these two factors may also interact. Discrimination abilities may change in response to the relative frequency of two interbreeding species. The underlying reason appears to be that male pied flycatchers have a song that incorporates more elements of the song characteristics of male collared flycatchers into their own song repertoires when occurring in areas inhabited predominantly by collared flycatchers. I investigated selection pressures acting on hybrids. In migratory species, hybrid fitness might be reduced as a consequence of intermediate suboptimal migration routes (extrinsic post zygotic isolation). Comparison of stable isotope signatures of revealed that parental species have separate wintering grounds, but hybrids appear to winter at the same location as pied flycatchers. A possible dominance effect in the inheritance of migration direction may hence reduce this potential cost. This interpretation is supported by the absence of a reduction in juvenile to adult survival of hybrids. By further comparing male hybrid fitness to that of the parental species, using lifehistory data, I demonstrate that hybrid males experience a moderate reduction in fitness (mainly through a sexually selected disadvantage). Sexual selection acting on male hybrids can play a major role in the speciation process because when the same characters affect assortative mating as well as hybrid fitness, reinforcement of reproductive barriers becomes more likely. Even when reproductive isolation is completed- the fate of newly formed species may be uncertain since they may strongly compete for ecological space. Long-term persistence of ecologically similar, species requires that there are spatial or temporal variation in their relative fitness. The growth of nestling pied flycatchers is less affected by harsh environmental conditions. We suggest that a regional co-existence of the two flycatcher species is due to a lifehistory trade-off between interference competitive ability and robustness to a harsh conditions. Overall, the studies in this thesis reveal the complexity of the interactions between mate choice and competition in shaping sexual signals. Furthermore, it suggests that natural selection is moderate on hybrid males and that sexual selection may have strong implications for the maintenance of species integrity.

Keywords: postzygotic isolation, prezygotic isolation, asymmetrical isolation, sexual signals, hybrid fitness, co-existence

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List of Papers

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


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Contribution
In chapter I, I contributed to the planning, execution of the experimental part and the writing process. In chapter II, I carried out all stages of the process except extracting sound waves. In chapter III, I share first authorship with Thor Veen, we both planned and contributed equally in analysis and writing. In chapter IV, I carried out all stages of the process. In chapter V, I contributed to planning, execution of the experimental part and the writing process.
The picture on the cover was drawn by Tomas Pärt
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Introduction

Hybridization

Hybrid zones can arise for several reasons, but there are two main patterns that can be distinguished. Firstly, the cause of hybrid zones can be entirely ecological. For instance, in a heterogeneous environment, different populations may adapt to different conditions and in the boundaries between different habitats, intermediate hybrids can form (hybrid zones). However, the degree of hybridization is dependent on both time since divergence (time for adaptation) and distinct ecological differences between habitats. Secondly, hybrid zones can be formed when two allopatric populations come into secondary contact and reproductive isolation is incomplete. This may happen as a result of natural changes in the distribution of species. However, as a result of direct (i.e. introductions of alien species) or indirect (i.e. changes in climate or habitat structure) human impact, species that have been separated for tens of thousands of years are now being brought together at a very high speed (Mooney & Cleland 2001). When pre-zygotic isolation is incomplete between populations, it may lead to heterospecific pairings and this may have severe detrimental effects on the maintenance of native species (Levin et al. 1996, Rhymer & Simberloff 1996, Mooney & Cleland 2001, Mallet 2005). Patterns of hybridization, and especially patterns of backcrossing, are difficult to detect and evaluate in natural populations (Rhymer & Simberloff 1996, Mallet 2005). Thus, the implications of heterospecific pairing and backcrossing patterns are poorly known.

Although natural hybridization in general is rare, it is a fairly common phenomenon in some taxa e.g. in frogs (Sumida & Ishihara 1997), butterflies (Turner 1971), grasshoppers (Barton & Hewitt 1985) and fish (Billington & Hebert 1991). Since speciation can be viewed as the build up of reproductive barriers between populations, a major expectation is that the more closely related two species are, the more likely they are to hybridize (Mayr 1942). Further, hybridization is expected to occur when species have spent relatively less time in sympatry. This second scenario is based on the theory of reinforcement; when hybrids experience a reduction in fitness, natural selection favor traits that reinforce reproductive barriers between species.
Due to the rare occurrence of natural hybridization and difficulties of measuring fitness in the natural environment, most studies have been performed using domesticated or laboratory species (Rhymer & Simberloff 1996, Mallet 2005). However, investigating the causes and consequences in natural populations would provide valuable insights on the relative role of hybridization in driving local extinctions. Furthermore, such studies would generate important knowledge into the mechanisms behind the evolution of reproductive isolation.

Why hybridize?

The occurrence of hybridization is generally attributable to species densities and to the degree of differentiation in mating behavior between two parental populations. When individuals have problems finding a conspecific mate, they might have to face the option of engaging in heterospecific pairing, which may be better than not to reproduce at all. In fact, hybridization is more often reported when at least one of the parental species is rare (Randler 2002). In paper I, we isolate the effect of species rarity per se by comparing the same species pair under different population structures. Another important reason for hybridization may be that individuals have poor discriminatory abilities against heterospecific mates. This situation is plausible if sexual signals and/or mate preferences are not sufficiently diverged between two closely related species. However, divergence in sexual signals may in some animals constitute the only barrier to gene flow between populations (Grant & Grant 1997). One very interesting aspect of the evolution of reproductive isolation, is, what role culturally transmitted sexually selected traits (i.e. vocal cues) play in the speciation process. Such signals have the potential to both quickly diverge (West-Eberhard 1983, Grant & Grant 1997, Price 1998, Irwin et al. 2001) and converge (Qvarnström et al. 2006) between populations. Therefore, the influence of socially transmitted sexual signals on reproductive isolation may be strongly context-dependent. In paper II, I investigate whether the effect of a partially learnt sexual signal (i.e. song) on reproductive isolation, is dependent on the relative frequency of the two parental species.

Implications of hybridization

Natural selection has generally been the underlying explanation to population variation and has, in the past, been the major interest in evolutionary biology. Adaptations to a novel or changing habitat can lead to reproductive isolation between populations as a by-product (Dobzhansky 1937, Mayr
Adaptive radiation in beak size and shape in the Darwin’s finches (Grant 1996), and the large diversity of trophic levels used by African cichlids (e.g. Schluter 1992, 1996), provide striking examples from nature. Artificial experiments performed by Rice & Hostert (1993) and Orr & Smith (1998) imitating natural selection, have shown to cause rapid evolutionary divergence of lineages. Once populations have diverged genetically, hybridizing individuals may experience a cost as a consequence of reduced hybrid viability and/or fertility (i.e. Hewitt 1989, Tegelström & Gelter 1990). In paper III, we investigate whether hybrids suffer the potential ecological cost of an intermediate migration route (extrinsic postzygotic isolation).

In contrast to natural selection, the role of sexual selection in speciation has been given less attention. Because sexual selection is thought to involve runaway processes, and acting directly on traits involved in mate choice, it can potentially produce rapid divergence of sexual traits that are important for pre-mating isolation (West-Eberhard 1983, Panhuis et al. 2001, Coyne & Orr 2004). Today, understanding the relative role and potential interactions between natural and sexual selection is one of the main goals in studies of speciation (Coyne & Orr 1998). Even though implications of sexual selection on prezygotic isolation are relatively established, its role in postzygotic isolation is rarely considered. This is unfortunate as postzygotic isolation barriers may also be the result of sexual selection. For instance, intermediate phenotypes of hybrid individuals might make them unattractive (i.e. intermediate plumage characters or song differentiated song repertoire) to potential mates of the parental species. And hence, may also increase the likelihood of reinforcement. As mentioned earlier, reinforcement (Dobzhansky 1937, 1940) is the process whereby reproductive barriers are formed as a result of natural selection against hybrids. The reinforcement theory has been highly controversial (e.g. Servedio & Noor 2003) and one of the main obstacles for reinforcement is gene flow. For instance, gene flow between different populations might swamp the effects of selection and recombination can break up important associations between the genes that affect both prezygotic isolation and postzygotic isolation (e.g. Sanderson 1989). In general, hybrids are assumed to have lower fitness then that of the parental species and in most cases, hybrid fitness is decreased because of genetic (i.e. developmental problems) or behavioral problems (i.e. intermediate sexual display). However, when selection against hybrids is driven through their unattractiveness, the same trait is involved in both pre- and post zygotic isolation. Hence, these kind of single-allele models of reinforcement (and sympatric speciation) avoid the problems with recombination and are therefore more likely to succeed (Servedio & Kirkpatrick 1997).

However, hybrids do not always suffer from large reductions in fitness. If hybrids have similar fitness to that of the parental species you would expect
that the two species merge into one large interbreeding population (Ferdy & Austerlitz 2002). In some rare cases, hybrids may even have higher fitness than the parental species (e.g. Barton 2001, Burke & Arnold 2001). This could be expressed through benefits associated with increased heterozygosity, but it may also be that hybrids have neither a fitness advantage nor disadvantage (Grant & Grant 1992). Furthermore, interbreeding may still be evolutionary important since it produces novel combinations of genes (e.g. Burke et al. 1998) which in turn might lead to rapid evolutionary changes such as speciation (Grant & Grant 1992).

Studies on the relative importance and interaction between natural and sexual selection on hybrids can give important insight into the process of population divergence and for predicting the future evolution of interbreeding populations. The most informative approach is to measure the relative difference in fitness between hybrid and parental species under natural conditions. In paper IV, I hope to provide a more extensive framework of the forces of natural and sexual selection acting on hybrids.

Co-existence of closely related species

The likelihood of two species hybridizing increases with their phylogenetic relatedness, and, the likelihood that they will compete over similar resources increases as well. Traditionally, interspecific competition over similar resources was thought to result in either niche differentiation or extinction of the poorer species (Volterra 1926, Lotka 1932). Hence, newly formed sister species were not expected to be able to live in sympatry if they had not evolved to become adequately ecologically differentiated. Recent models suggest that co-existence of ecologically similar species is favored if fluctuations in the environment favor different species at different times or places (spatial and temporal heterogeneity) (see e.g. Chesson & Warner 1981, Chesson & Huntley 1997, Amarasekare & Naisbet 2001). Even so, it is not intuitive that species that utilize similar resources would react differently to fluctuations in the environment. In paper V we investigate whether differences in lifehistory traits (i.e. differentiated resource allocation) may facilitate co-existence of two ecologically similar species.
Study Species and Methods

Study Species
Collared (*Ficedula albicollis*) and Pied flycatchers (*F. hypoleuca*) are two closely related species of migrating, passerine birds that hybridize where they co-occur in central and eastern Europe and on the two Baltic islands Öland (57° 10´N, 16° 58´E) and Gotland (57° 10´N, 18° 20´E), off the east coast of Sweden (Alatalo et al. 1990). The two species are thought to have come into secondary contact after the last glaciation period (Saetre et al. 2001). The Baltic hybrid zone is relatively young, where the two *Ficedula* species have been in contact for approximately 150 years (*F. hypoleuca* being the predecessor). On the two Swedish islands, the densities of the two species are greatly skewed, where collared flycatchers outnumber pied flycatchers on both Öland and Gotland.

The males arrive at the breeding grounds approximately a week earlier than females (Pärt & Gustafsson 1989) and start to defend a territory and females visit several males before settling (Dale et al. 1990). Males do not appear to discriminate between females of the two species but females are shown to mate assortatively (Saetre et al. 1997).

The data presented in thesis is collected from populations of collared and pied flycatchers on the Swedish island of Gotland and Öland. This *Ficedula* population has been studied rigorously for over 25 years. On Gotland, nest box areas have been established since the 1980’s up until today (rendering around 3000 nest boxes). On Öland, nest box areas have been established in two separate parts, from 1981-1985 and 2001-2006 (rendering around 2500 boxes). The sizes of the nest box areas vary from ca 15 boxes up to 500 boxes.

Data from all breeding attempts are collected through the entire breeding season (late April to mid July). All nest boxes are visited at regular intervals during the breeding season and data on pair formation, lay date (day of first egg), clutch size, number of hatchlings and fledglings is recorded. Females are typically caught when incubating the eggs and males are caught when arriving to feed nestlings. Individuals are usually caught inside the nest box (using flap traps) or just outside (using mist nets). All individuals are ringed and morphological measurements are taken (i.e. tarsus length, wing length
and size of forehead patch, wing patch and white in tail feathers). When
nestlings are 12 days old, they are ringed, measured and blood samples are
taken from all individuals.

Main Results and Conclusions

Asymmetrical hybridization (paper I)

In natural hybrid zones, it is often observed that one of the two species is
rare (e.g. Randler 2002) and that this rarity might promote hybridization and
possibly result in asymmetrical hybridization (Wirtz 1999). Asymmetrical
hybridization represent the case when the female in heterospecific pairs
more often belong to the rare species than expected by chance. This is be-
cause females are only expected to hybridize when conspecific mates are
rare, while males are expected to be less discriminatory. However, it is also
important to acknowledge that not only is hybridization is not only influ-
enced by the relative availability of conspecific individuals, but also by the
strength of the preferences for species specific traits (Panhuis et al. 2001).

In paper I, using simulations and experimental song broadcast experiments,
we investigate the effects and interactions between discriminatory ability of
males and females and the effects of limiting conspecific mates on the rate
and direction of heterospecific pairings.

As expected, we found that female pied flycatchers were more likely to en-
gege in heterospecific pairings in sites where conspecifics were rare (Figure
1), but, the overall hybridization rate was highest in sites were the two spe-
cies were at equal frequencies (Figure 2a). Our simulations suggest that this
pattern might arise when there are moderate levels of error in species recog-
nition. Therefore, both a lack of conspecifics and error in species recognition
are important factors explaining hybridization in flycatchers. Further, there
was an overall bias towards pied females being involved in heterospecific
pairings and the results from the song broadcasting experiment indicates that
pied flycatchers are less discriminatory when responding to the two specie
specific song-types (figure 3). We found the rather unexpected result that the
bias towards female pied flycatchers in heterospecific pairs increased when
pied flycatchers became less rare (figure 2b).
Figure 1. The effect of relative abundance on the hybridization risk of individual females of either species. Filled squares and the solid line refer to female pied flycatchers and open squares and the dotted line refer to female collared flycatchers.
Figure 2. Simulations showing the expected relationship between relative abundance and (a) the overall hybridization rate within a forest site, and (b) the bias in the direction of hybridization towards heterospecific pairs with female pied flycatchers.
This result implies that rarity somehow affect the error rate of choosy females either through plasticity in their own behavior or through plasticity in male sexual signals in response to the relative frequencies of the two species.

Vocal Plasticity (paper II)

In paper II, I test the possibility of a plastic response in a male sexual signal to the relative frequencies of the two species. I found that male pied flycatcher change their song according to the social environment. A principal component analysis (PCA) shows that pied males have a song that incorporates more of the collared male song characteristics when occurring in areas inhabited predominantly by collared flycatchers (Figure 1). PC1 is significantly different between pied males in areas where the abundance of collared flycatchers is low or relatively equal to pied flycatchers. However, PC1 is not significantly different between collared males and pied males occupying territories in areas predominantly inhabited by collared flycatchers.

**Figure 3.** Number of female (top panel) and male (lower panel) collared and pied flycatchers attracted to broadcasts of song from conspecifics and heterospecifics.
Figure 4. Principal component analysis of the songs of males from the four different site-groups. “Pied A” (white circles) are males in areas with predominantly pied flycatchers, “Pied B” (grey circles) are males in areas with an equal proportion of the two species, “Pied C” (black circles) are males in areas with predominantly collared flycatchers and “Collared” (black triangles) are male collared flycatchers from areas inhabited predominantly by collared flycatchers.

Thus, male pied flycatchers appear to adjust their song repertoire in response to the local relative abundance of collared flycatchers. The fact that the females in heterospecific pairs more often is a collared flycatcher (in areas where collared flycatchers are relatively common; paper I) might therefore be explained by male pied flycatchers being less reliable when signaling their species identity in such areas. More precisely, the song of pied males is less reliable as a species-specific mate recognition cue in areas where collared flycatchers are more abundant (pied males sing more similar to collared males). Plasticity in sexual signals might therefore have important context-dependent effects on reproductive isolation between closely related species. Vocal plasticity may under certain conditions, facilitate heterospecific pairings and hence, reduce reproductive isolation between collared and pied flycatchers.
Migration pattern of male hybrids (Paper III)

When heterospecific pairing is evident, species integrity can only be maintained through postzygotic isolation barriers (Coyne & Orr 2004). Postzygotic isolation barriers need not only be intrinsic (i.e. hybrid sterility), but may also come about through extrinsic factors (i.e. intermediate phenotype leading to reduced fitness). Hybrids may as a result of their typically intermediate phenotype, fall in between parental niches and therefore suffer from a reduction in fitness (Coyne & Orr 2004). Recently, Sutherland (1998) suggested that in migratory passerine birds, a suboptimal migration route potentially taken by hybrids may enhance postzygotic reproductive isolation.

In paper III, we investigate whether possible differences in migration routes are acting as a potential extrinsic isolation barrier between pied and collared flycatchers. The locations of wintering sites of collared and pied flycatchers were previously largely unknown. Observations of ringing recoveries are scarce but there are a few findings (ring recoveries and visual observations) suggesting that the wintering locations differ between the two species (Cramp 1992, Lundberg & Alatalo 1992). In this study we used stable isotopes of Carbon and Nitrogen (extracted from feathers molted at the wintering grounds), to be able to characterize the wintering locations of collared and pied flycatchers and their hybrids.

We found that pied and collared flycatchers, as well as hybrids show a clear difference in their $\delta^{13}$C distribution. This pattern is explained by pied and collared flycatchers differing in $\delta^{13}$C while hybrids had $\delta^{13}$C values similar to that of pied flycatchers. There was no difference in $\delta^{15}$N values between parental species or hybrids. Also, the $\delta^{13}$C stable isotope distributions of hybrid individuals suggest that hybrids (regardless of parental species pairing) fall within in the distribution range if pied flycatchers (figure 5).
We argue that this result is an effect of pied migration route being dominant. By following one of the parental migration routes and using their wintering grounds, it appears that hybrid individuals do not bear the costs of a potential suboptimal migration route. Also, we found that juveniles from heterospecific pairs have similar survival rates as that of collared flycatchers. Hence, we conclude that the potential dominance effect can reduce extrinsic postzygotic isolation between collared and pied flycatchers.

Natural and Sexual Selection on male hybrids (paper IV)
The study of lifetime reproductive success of hybrids provides important insights into the different mechanisms of the evolution of reproductive isolation. In paper IV, we investigate different sources of natural and sexual selection acting on male hybrid flycatchers. We investigate lifetime reproductive success of hybrid males by following the different aspects of their life cycle. More specifically, we investigate several features such as survival to breeding, pairing success, clutch size, proportion of eggs hatched, proportion of extra-pair young in broods, proportion of hatchlings fledged, proportion of fledglings coming back to breed and life span. Table 1 summarizes the fitness components acting in different stages in the life cycle.
Table 1. Summary of the different fitness components acting at different stages in the life cycle of males. Lifetime reproductive success is estimated by factoring all fitness components. Relative fitness is estimated from the two parental species frequencies.

<table>
<thead>
<tr>
<th>Fitness components</th>
<th>CF</th>
<th>PF</th>
<th>HY</th>
<th>INTER</th>
<th>Primary force of selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prob. pairing</td>
<td>0.729</td>
<td>0.509</td>
<td>0.552</td>
<td>0.727</td>
<td>Sexual</td>
</tr>
<tr>
<td>Prob. eggs hatched</td>
<td>0.925</td>
<td>0.935</td>
<td>0.849</td>
<td>0.896</td>
<td>Natural</td>
</tr>
<tr>
<td>Prop. WPY</td>
<td>0.920</td>
<td>0.961</td>
<td>0.370</td>
<td>0.737</td>
<td>Sexual and Natural</td>
</tr>
<tr>
<td>Prop. hatchlings fledged</td>
<td>0.811</td>
<td>0.876</td>
<td>0.898</td>
<td>0.832</td>
<td>Natural</td>
</tr>
<tr>
<td>Prob. fledglings recruited</td>
<td>0.111</td>
<td>0.064</td>
<td>0.137</td>
<td>0.087</td>
<td>Natural</td>
</tr>
<tr>
<td>Life span</td>
<td>2.308</td>
<td>2.750</td>
<td>2.792</td>
<td>1.833</td>
<td>Natural</td>
</tr>
<tr>
<td>Lifetime repr. Success</td>
<td>0.786</td>
<td>0.444</td>
<td>0.367</td>
<td>0.388</td>
<td></td>
</tr>
<tr>
<td>Relative fitness</td>
<td>1</td>
<td>0.565</td>
<td>0.467</td>
<td>0.494</td>
<td></td>
</tr>
<tr>
<td>Success at sexual selection relative to CF</td>
<td>1</td>
<td>0.752</td>
<td>0.612</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Success at natural selection relative to CF</td>
<td>1</td>
<td>0.750</td>
<td>0.763</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results indicate that male hybrids have approximately half the fitness of male collared flycatchers. This large reduction in reproductive success is mainly explained by a low pairing success and very high ratios of extra-pair paternity found in broods raised by hybrid males. However, the level of postzygotic isolation against male hybrids seems to be mediated both through female mate choice and cryptic isolation (i.e. sperm incompatibilities).

When estimating the relative effects of natural and sexual selection, we found that overall, sexual selection against hybrid males accounts for approximately a 38.8% reduction in fitness, while natural selection accounts
for a 23.7% reduction. These estimates suggest that reduced sexual attractiveness of hybrid males can play a major role in postzygotic isolation between the two flycatcher species. Furthermore, we suggest that reduced fitness of hybrid males, caused by a considerable sexual selected disadvantage, may have important effects on speciation because when the same characters affect assortative mating as well as hybrid fitness, further reinforcement of reproductive barriers is becomes more likely.

Co-existence (paper V)

Traditionally, interspecific competition over similar resources was thought to result in either niche differentiation or extinction of the poorer species (Volterra 1926, Lotka 1932). Recent models suggest that co-existence of ecologically similar species is favored if fluctuations in the environment favor species at different times or places (see e.g. Chesson & Warner 1981, Chesson & Huntley 1997, Amarasekare & Nisbet 2001).

In the *Ficedula* system, collared flycatchers are dominant over pied flycatchers in competition over preferred nest sites (Lundberg & Alatalo 1992). Hence, pied flycatchers seem to be out-competed in areas where they co-occur (Gustafsson & Pärt 1991, Saetre et al. 1999). However, Saetre et al. (1999), suggest that collared flycatchers higher sensitivity to large-scale climatic variation, might counteract the competitive exclusion of pied flycatchers (Saetre et al. 1999). One hypothesis that might explain this pattern is niche differentiation (i.e. males of the two species occupy different microhabitats where resource availability differ). Alternatively, the two ecologically similar species use largely the same resources but, offspring intrinsic abilities in allocating resources differ between the two species. This might lead to different responses of the fitness of the two species in a changing environment.

In paper V, we use long term data from collared and pied flycatchers to investigate co-variation of life history traits (e.g. number of offspring produced and nestling growth) and the onset of breeding. In addition, we performed a field experiment where we cross-fostered three day old nestlings (matched for hatching dates) between the two species to be able to control for possible differences in niche use by the parents.

We found that collared and pied flycatchers differ in their reproductive strategy and in their response to a stressful environment (interpreted as declining environmental conditions during the season). The collared flycatchers seem to be more sensitive to harsh environments than pied flycatchers. Collared flycatchers have significantly lower reproductive success compared to pied
flycatchers later in the season (the relationship between lay date and fledgling success is shown in Figure 6).

![Figure 6](image)

**Figure 6.** Reproductive success of collared and pied flycatchers in relation to the timing of breeding. Open squares represent collared flycatchers and filled squares represent pied flycatchers. Collared flycatchers experience a significantly steeper seasonal decline in reproductive success than pied flycatchers.

In the cross fostering manipulation we could investigate the effect of breeding time *per se* by comparing the relative fitness of nestlings (siblings and foster siblings) reared in the same nest (i.e. controlling for potential differences in niche use by the parents). We analyzed weight differences between nestlings at day three (day of brood swap) and weight at fledgling (day 12).

Nestlings of pied flycatchers are less sensitive to the seasonal decline in environmental conditions (Figure 7). Even though the collared flycatchers are the dominant species in the *Ficedula* system, the growth of their young is relatively more sensitive to harsher conditions later in the breeding season. We suggest that a lifehistory trade-off between investment in interference competition and robustness to a harsh environment explains the overall distribution pattern of the two flycatcher species.
Figure 7. Difference in mean weight at fledgling between nestling collared and pied flycatchers being raised in the same nests in relation to timing of breeding. Open squares represent nests attended by collared flycatchers, and filled squares represent nests attended by pied flycatchers. Mean breeding dates are indicated by open and closed arrows for collared and pied flycatchers respectively. Nestling collared flycatchers experience an advantage when reared early in the season and when reared by pied flycatchers.

Conclusions and Future prospects

Behaviorally driven sexual isolation between animal species is known to depend on both the availability of conspecific mates and on the discrimination ability of individuals. The results from my thesis demonstrate that these two factors may also interact. The discrimination ability may change in response to the relative frequency of two hybridizing species. In this case, the underlying reason appears to be that male pied flycatchers have a song that incorporates more of the collared male song characteristics when occurring in areas inhabited predominantly by collared flycatchers. Therefore, future studies on prezygotic isolation need to address the possibility that plasticity in male sexual signals may lead to context-dependent species recognition.

When reproductive isolation is incomplete, investigating the effects of natural and sexual selection acting on hybrids, will give insight into the processes
that influence population divergence. Natural selection is typically recognized as the main agent of selection against hybridization (i.e. hybrids falling between parental niches). However, postzygotic isolation can also be attributable to sexual selection. Today, the knowledge of the different mechanisms underlying selection regimes against hybridization is relatively unknown, but my results provide a step in that direction. We have shown that by following one of the parental species migration routes, hybrid flycatchers appear to avoid the potential risk of reduced survival (by taking an intermediate suboptimal migration route). In the *Ficedula* system, natural selection is the main agent against hybridization because hybrid females are sterile. However, the situation is more complicated for hybrid males. By a detailed comparison of natural and sexual selection on male hybrids, I found that a sexually selected disadvantage, experienced by hybrid males, seems to be a major force underlying postzygotic isolation between the two flycatcher species. This is caused by the low pairing success and higher ratios of extra pair young of hybrid male. Hence, sexual selection on male hybrids can facilitate reinforcement of premating isolation because the same characters effects assortative mating as well as hybrid fitness.

Interspecific competition between species with similar ecological needs is traditionally thought to lead to extinction of the subdominant species or niche differentiation. However, spatial or temporal variation in relative fitness may promote co-existence. We found that the growth of nestling pied flycatchers is less affected by the seasonal decline in environmental conditions. We suggest that co-existence of the two species is attributable to a lifehistory trade-off between interference competitive ability and robustness to a harsh environment. One interesting question then becomes: How will large-scale climatic changes influence the relationship between collared and pied flycatchers?

To summarize, this thesis hopes to have contributed with insights into the speciation process by pinpointing causes and consequences of hybridization. One of my major conclusions is that natural and sexual selection interacts during the whole speciation process.
Effekter av naturlig och sexuell selektion på hybridiserande flugsnappare.


Genom att studera vilka beteenden och situationer som leder till hybridisering mellan de båda flugsnappararterna, kan man dra generella slutsatser om vilka evolutionära förändringar som måste till för att genflödet ska avstanna. Med andra ord, vad krävs för att de båda arterna ska bli helt reproduktivt isolerade från varandra? Resultaten från de två första kapitlen i avhandlingen


Genom att ha en intermediär fenotyp kan hybrider ställas inför en hel del ekologiska problem. Ett sådant tänkbart problem hos flyttfåglar är ifall de båda föräldra-arterna flyger till olika övervintringsplatser och hybriderna ärver en "intermediär" färdriktning och hamnar helt fel, kanske mitt i öknen. Vi har visat (kapitel III) att svartvit och halsbandsflugsnappare övervintrar på olika platser men deras hybrider verkar följa en av föräldra-artens flygång under migrationen (dvs. undvik en intermediär, mindre optimal flygång). I en annan mer detaljerad studie undersökte jag flera olika aspekter i hybridhannars livscykler, såsom panningsframgång, antal flygga ungar, livslängd mm. I denna studie jämförde jag naturligt och sexuellt selektionstryck på
hybridhannar, och fann att sexuell selektion verkar ha den största påverkan på hybridhannars fitness (kapitel IV). Detta visades genom att hybridhannarna inte har lika stor framgång i att attrahera honor samt att största andelen av den avkomma som hybridhannarna uppfostrar, i själva verket är avkomma till en annan hanne.

När två arter är närbesläktade, lever i samma habitat och har liknande ekologiska behov, har det tidigare föreslagits att den ena arten (den som är sämre på att konkurrera), kommer att dö ut om den inte utvecklar sin egen niche. De båda flugsnappar-arterna konkurrerar om samma typ av bohål och mat (insekter). Emellertid kan spatial och temporal variation (vilken art som är "bäst" i vilken situation och tid), tillåta att de två konkurrerande arterna kan fortsätta att samexistera. Vi fann att avkomman till svartvita flugsnappare var mindre känsliga för omgivningens förändringar underhäckningssäsongen. Halsbandsflugsnappare är däremot bättre på att konkurrera om bohål. Därför föreslår vi att de båda arterna kan samexistera på regional nivå på grund av en avvägningsskillnad (trade-offs) mellan konkurrensförmåga och förmåga att motstå förändringar i omgivningen; halsbandsflugsnapparna har satsat på att vara dominant i direkta konfrontationer (slagsmål om holkar), medan svartvit flugsnappare är bättre på att klara av tuffa väder och habitat förhållanden.

Resultaten från min avhandling presenterar goda bevis för att sexuell selektion kan ha konsekvenser för evolutionen av postzygotiska reproduktiva barriärer mellan närbesläktade arter.
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