Speciation - What Can be Learned from a Flycatcher Hybrid Zone?

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Abstract

Studies of hybrid zones offer important insights into the process of speciation. Much of the knowledge to be gained is dependent on an accurate estimation of the strength of pre- and post-zygotic isolation between hybridizing taxa. My results demonstrate that hybridization can variously affect different components of fitness. In Ficedula flycatchers, late-breeding females may directly benefit from pairing with a heterospecific male by gaining access to superior territories. The hybrid offspring possess an immune system that is as equally well functioning as in the parental species (the collared, F. albicollis, and pied flycatcher, F. hypoleuca). However, I found that a severe reduction in fertility persists for at least three generations after the actual hybridization event. Combining all information about the reproductive success of hybridizing individuals and their descendents revealed that postzygotic isolation between flycatchers is very strong; hybridizing individuals leave almost no descendents. This thesis presents one of few comprehensive summaries of the selection for/against assortative mating in a natural hybrid zone. These findings suggest a central role for intrinsic postzygotic isolation as a reproductive barrier separating newly evolved bird species, and contrast previous suggestions that postmating isolation is the slowest of the reproductive barriers to evolve in birds.

Despite this strong selection against hybridization, pre-mating isolation is incomplete. Hybridization often results from females lacking conspecific partners, but appears to be also caused by errors in species recognition. Much of this error probably reflects the short period of time that pied flycatchers on Gotland and Öland have been in sympathy. Compared to collared flycatchers, pied flycatchers are poorer able to discriminate between conspecific and heterospecific song, and male pied flycatchers more often falsely signal their own identity through heterospecific song copying. However, despite colonising the study site from other sympatric populations and having very little gene flow from allopatry, collared flycatchers also possess traits (e.g. delayed plumage maturation) that increase their hybridization risk. Once pre-mating isolation is strong, the rarity of hybridization probably inhibits further selection against traits promoting interspecific mating, especially when such traits may be beneficial in other contexts. This thesis highlights complex interactions between factors affecting hybridization rate that would not be detected if such a study were not field-based. Furthermore, it showcases likely examples in nature of a number of theoretical objections to the evolution of pre-mating barriers between populations living in sympathy.

Keywords: speciation, reinforcement, fitness, postzygotic isolation, asymmetrical hybridization, hybrid zone, species recognition, mate choice, sexual selection, direct benefits, parasite resistance, delayed plumage maturation, Ficedula hypoleuca, Ficedula albicollis, flycatcher

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List of papers

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


II  Wiley, C., Qvarnström, A. & Gustafsson, L. Effects of hybridization on the immunity of flycatchers and their infection by blood parasites (submitted).


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For each of the chapters included in this thesis, I was heavily involved in the planning of experiments and the collection of field data, performed much (III) or all (II) of the molecular work, performed all of the statistical analyses (except the assignment of genotypes in III), and prepared all manuscripts. In all papers, co-authors contributed intellectually to the planning of experiments and final stages of manuscript preparation, as well as with field data and laboratory work (III only).
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### Abbreviations

<table>
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<tr>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>PHA</td>
<td>Phytohaemagglutinin</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<tr>
<td>F(_1)</td>
<td>First-generation hybrid</td>
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Introduction

The enormous diversity of life on Earth faces us with a number of important and challenging questions. How have all these species arisen? What barriers prevent incipient species from interbreeding with each other? And why aren’t new species out-competed by the old? Many of these questions are not merely philosophical, but also dictate how we should manage this biodiversity in a way that preserves the variation as well its potential for self-perpetuation. However, such fundamental questions are by no means easily answered, owing largely to the timescales over which the process of speciation typically occurs. For these reasons, despite the subject of speciation having attracted considerable attention from the scientific world, our understanding of the process remains fragmentary (Coyne & Orr, 2004).

Natural hybrid zones, geographical regions where closely related species meet and occasionally interbreed, provide important insights into the evolutionary processes characterizing the final stages of speciation. This is because, by focusing on young pairs of sister-species, we can better understand the types of reproductive barriers that are important for speciation to occur. For example, we can examine the relative strengths of post- and pre-zygotic isolation between populations that maintain their integrity in sympatry versus those that fuse. We can also investigate how post-zygotic isolation (i.e. unfit hybrids) influences the evolution of pre-zygotic isolation (species recognition). Overall, hybrid zones provide us with a valuable tool for investigating the conditions favouring the evolution of new species, and allow us to test predictions from theoretical models of speciation in nature.

Although interspecific hybridization is a widespread phenomenon (e.g. Johnson, 1939; Grant & Grant, 1992; Dowling & DeMarais, 1993), by definition it is infrequent. Populations are defined as different species when gene flow between them is sufficiently small that each remains distinct (modified version of Mayr’s [1995] biological species concept, as advocated by Coyne & Orr [2004]). Unless there are severe post-zygotic barriers to reproduction (e.g. hybrid sterility), gene flow between populations through hybridization ultimately leads to the breakdown of reproductive barriers and fusion into a single species (Felsenstein, 1981). On the other hand, in the face of severe post-zygotic isolation, individuals evolve to mate only with their own species (a process termed reinforcement) (Dobzhansky, 1937; Blair, 1955), thus reducing the frequency of hybridization. However, it is this transitory nature
of many hybrid zones that makes them ideal for studying the conditions under which speciation is prevented or completed.
The *Ficedula* hybrid zone

Pied (*Ficedula hypoleuca*) and collared flycatchers (*F. albicollis*) are both well-studied model organisms, and much is known regarding their breeding biology and reproductive isolation. Because of limited space within each chapter of this thesis for summarizing this knowledge, I have attempted to outline important aspects of their biology in some detail here.

Pied and collared flycatchers are small, short-lived, migratory passerine birds that breed in mixed and deciduous forests in Europe. They winter in sub-Saharan Africa, and arrive at the breeding grounds in late April or early May. Soon after arrival, males aggressively occupy and defend breeding holes (Alatalo et al. 1994; Qvarnström 1997), and females subsequently inspect a number of males/territories before selecting their partner (Dale and Slagsvold 1996). Their ready acceptance of nest-boxes makes them ideal study objects. Females typically lay five to seven eggs, and are the sole incubator (when they are caught and measured). Both sexes assist in the feeding of nestlings (when males are caught and measured), and only a single brood is produced each year. Monogamy prevails, although at least 9% of males are polygynous (Qvarnström & Gustafsson, 2006), and approximately 15% of nestlings are sired by extra-pair males (Sheldon & Ellegren, 1999).

The two species probably remained geographically isolated in separate glacial refuges during the Pleistocene, and have since expanded their breeding ranges northward (Sætre et al., 2001). Today, they co-occur (are sympatric) within a broad hybrid zone that includes much of central and eastern Europe, with an isolated hybrid zone on the Swedish islands of Gotland and Öland in the Baltic Sea. The two species have remained morphologically distinct in the face of hybridization. The work included in this thesis was carried out within the Swedish hybrid zone, which constitutes a more recent contact between the two species (around 100 years) (Lundberg and Alatalo, 1992). On the Swedish islands (as in much of Europe), collared flycatchers have gradually expanded their range, excluding pied flycatchers from optimal breeding habitats (Alatalo et al., 1982, 1994; Sætre et al., 1999; see Fig 1). The Swedish hybrid zone is surrounded on all sides by allopatric pied flycatchers, which is a likely source of migrants into the hybrid zone. Immigration into the Swedish collared population, when it occurs, most likely involves individuals arising from other hybrid zones in eastern Europe.
Figure 1. (A) Breeding distribution of pied (red), collared (yellow) flycatchers. Hybrid zone is shown in orange (adapted from Haavie et al., 2004). (B) Typical mixed forest inhabited by pied flycatchers. (C) Typical, high quality, deciduous forest dominated by collared flycatchers (both B and C taken in northern Öland). (D) Location of nest-box areas monitored within the Swedish flycatcher hybrid zone.

Figure 2. (A) Adult male collared flycatcher, (B) subadult male collared flycatcher, (C) male hybrid, and (D) male pied flycatcher. Photos kindly provided by N. Svedin and M. Hjernquist.

The males of both species have bold, black-and-white plumage patterns, which are involved in intraspecific sexual selection (both male-male compe-
tition and female choice) (Pärt & Qvarnström, 1997; Qvarnström, 1999; Qvarnström et al., 2000). However, they delay maturation of these traits until they are two years old. Subadults (one year old) are sexually mature, but are less boldly marked than older birds, either to avoid conflicts with dominant, older males (Qvarnström, 1997), or through developmental constraints. The two species differ in the size of their white patches and the blackness of their pigmented plumage, which not only permits their identification by humans, but may also be important for females to recognize conspecific mates (Sætre et al., 1997a). Females of the two species are both dull grey-brown, and differ only in subtle differences in the tone of their upperparts and the amount of white at the base of their neck feathers (Svensson 1992). Previous mate-choice trials have demonstrated that females display clear preferences for males of their own species (Sætre et al., 1997b). Males, on the other hand, appear to be less selective towards whom they display (Dale and Slagsvold 1994; Sætre et al., 1997b). Plumage, however, is unlikely to be the only cue used for mate-recognition, as the two species also have distinct songs. Song is thought to be an important sexual signal, isolating many species that otherwise do not differ in secondary sexual traits (Martens, 1996). However, the use of song by female flycatchers for locating conspecific mates is complicated by the fact that in areas of sympatry many male pied flycatchers learn to sing like collared flycatchers (Gelter, 1987; Alatalo et al., 1990; Haavie et al., 2004; Qvarnström et al., 2006). Whether this ‘mixed singing’ has evolved in males to signal territory occupancy to individuals of both species, or whether it is a maladaptive behaviour persisting from allopatry is currently unclear. However, mixed singing is associated with high rates of hybridization (Qvarnström et al. 2006).

The above-mentioned pre-mating barriers between the two species are quite strong. Heterospecific pairs typically constitute only 5.1% of the study population on Öland, and 2.4% on Gotland. When hybridization does occur, it results in the production of largely unfit F₁ offspring. Females are sterile, and F₁ males also have reduced fitness (Alatalo et al. 1990; Gelter et al. 1992; Veen et al., 2001). Postzygotic barriers acting on female hybrids are mostly intrinsic, although males appear to suffer mostly through their inability to compete for mates (sperm inviability is another likely possibility) (Svedin, 2006). F₁ hybrids are intermediate in morphology between the two parental species (Sætre et al., 2003). They frequently resemble collared flycatchers, although their white collar is incomplete, they have more grey in the plumage, and they possess little white in their primaries. However, past studies into the fitness of hybrids have been fraught with problems arising from the lack of genetic tools to clearly separate the different hybrid generations (i.e. F₁ and backcrosses). Furthermore, some aberrant collared flycatchers (especially subadults) closely resemble hybrids, and genetic tools are required to distinguish true hybrids. Prior to this thesis, nothing was
known about the fitness of later generation hybrids, a situation common to almost all natural hybrid zones. Despite the low fitness of hybrids, one previous study suggested that heterospecific pairing is sometimes adaptive (Veen et al., 2001). Female collared flycatchers can reduce the indirect costs of heterospecific pairing by copulating with extra-pair conspecific males and biasing the sex ratio of their offspring towards sons (Veen et al., 2001). Furthermore, the fact that such heterospecific pairs produce more fledged young (perhaps a result of direct benefits of heterospecific pairing) during the food-limited later half of the breeding season suggests that heterospecific pairing may be adaptive for late-arriving female collared flycatchers (Veen et al., 2001).
How maladaptive is hybridization?

Estimating the fitness of hybridizing individuals relative to those paired with conspecific mates not only provides a measure of the strength of selection against hybridization, but also of the reproductive isolation between sympatric populations. It is therefore important for predicting the amount of gene flow between two taxa, and is vital for inferring the likelihood that complete reproductive isolation will ultimately arise through reinforcement. When the $F_1$ hybrids produced are sterile, hybridizing taxa are fully reproductively isolated. Because gene flow is prevented, they can be considered separate species. Furthermore, there is expected to be strong selection on individuals to avoid interspecific mating (Dobzhansky, 1937), such that eventually the two species are expected to rarely, if ever, interbreed. However, when the resultant hybrids are not fully sterile or inviable, a small amount of gene flow results. Gene flow can effectively disrupt the process of reinforcement by preventing linkage disequilibrium between genes causing prezygotic isolation and those causing postzygotic isolation, leading to an amalgamation of the two genomes (Barton & Hewitt, 1981; Felsenstein, 1981). Whether low levels of gene flow prevent reinforcement has been the topic of much theoretical discussion, and depends greatly on the details of the models employed to test this (Liou & Price, 1994; Kelly & Noor, 1996; Servedio & Kirkpatrick, 1997; Kirkpatrick & Servedio, 1999; Servedio, 2000). This is perhaps a question that is best answered by empiricists. Surveying the strengths of selection against hybrids in hybrid zones where species-boundaries easily break down versus those in which the two taxa remain distinct and display character displacement would provide the best tool for investigating the conditions favouring speciation via reinforcement. Unfortunately, there are very few systems for which we currently have good estimates for the strength of selection against hybridization (Arnold & Hodges, 1995; Rieseberg & Carney, 1998).

The main reason for this lack of knowledge stems not from a lack of work into hybrid zones, but from the difficulty of obtaining accurate estimates of fitness. Fitness is defined as an individual’s contribution to the gene pool of future generations. In practice, however, it is typically estimated through a range of indirect measures, without knowing how they reflect the true currency of fitness; the number of gene copies (descendents) in later generations (Benton & Grant, 2000). Such measures often include seed germination,
larval survival, body mass, growth rates, immunocompetence, success at attracting mates, or fertility. Situations where some fitness components are high and others are low in the same hybrids highlight the need for a holistic approach to estimating selection against hybrids.

*Ficedula* flycatchers highlight the problem with inferring selection from single fitness components. Initial investigations into the system revealed low fertility among hybrids (Gelter et al. 1992), which resulted in the conclusion that hybridization was maladaptive. The fertility of hybrids was 45.9% of that of pure individuals. However, a later study revealed that one combination of heterospecific pair (collared females × pied males) produced more chicks than conspecific pairs during certain times in the season (Veen et al., 2001), even though many of these were unfit hybrids. This suggested that there were either direct benefits to female collared flycatchers of hybridizing, or hybrids possessed enhanced vigour, which was only expressed in stressful environments. Either explanation represents a fitness component that acts to counter low fertility, at least to some extent. This raised some doubt over previous suggestions that hybridization was always maladaptive in flycatchers.

**Are there direct benefits of hybridization?**

In Chapter I, I explore the origin of the pattern that late-breeding heterospecific pairs often produce more offspring than conspecific pairs. In particular, I investigate whether females gain direct benefits from pairing with males of the other species. These direct benefits might include when heterospecific males possess superior territories, or utilize their territory in a way that benefits the nestlings (e.g. by providing more food or complementary food-types). I found that the two species fed similar items to nestlings, and that the proportion of the parental workload adopted by males was not different between the two species. Therefore, there were no indications that heterospecific males utilize their territories in ways that benefit the nestlings more than conspecific fathers. However, the territories occupied by heterospecific pairs did differ in quality from those occupied by conspecific pairs. The effects of territory quality and having heterospecific parents were disentangled by looking at the performance of nests in the same box as heterospecific pairs, but in other years. I found that late-breeding female collared flycatchers that pair with pied flycatchers have better territories than those that do not hybridize (see Figure 3). This pattern closely matched previously reported fledgling rates in nests reared by such heterospecific pairs (Veen et al, 2001).

Direct benefits of mate-choice are widely appreciated as an important component of intraspecific sexual selection (Thornhill, 1976; Searcy, 1979; Gwynne, 1984; Reynolds & Gross, 1990; Kirkpatrick & Ryan, 1991). De-
spite this, few studies have considered direct benefits to females of hybridization. Most, instead, focus on the indirect costs/benefits of producing hybrid offspring. This trend no doubt reflects the, often severe, nature of indirect costs of hybridization (e.g. sterility of hybrid offspring). Also, direct benefits can explain the evolution of intraspecific mate choice, while hybridization is normally viewed as maladaptive, and therefore unlikely to evolve in response to the existence of direct benefits. My findings do not argue against such logic. What they do suggest is that, despite the existence of indirect costs, there may be direct benefits to females of hybridization. Such benefits may be important in reducing selection against hybridization and thereby hindering the evolution of reproductive isolation. Furthermore, among hybrid zones there exists a continuum of postzygotic isolation, from complete sterility to hybrid vigour. In those hybrid zones where postzygotic isolation is weak, direct benefits of hybridization may dictate selection for /against hybridization.

Figure 3. Comparison of data on fledging success of heterospecific pairs with male pied flycatchers (left panel, curve 1: from Veen et al., 2001), and the quality of their territories (right panel, triangles: from Chapter I) with those of collared flycatchers (negative slope in left panel; squares in right panel). Heterospecific pairs tend to have better territories and produce more fledglings than pairs of collared flycatcher after two days before the mean laying date of each year.

In Chapter I, I also discuss another important aspect of speciation; namely, for the coexistence of two sister taxa, they must not only be reproductively isolated, but should diverge sufficiently in niche to prevent competitive exclusion (Gause, 1934; Lack, 1946; Macarthur and Levins, 1964, 1967). Collared and pied flycatchers prefer the same breeding habitat (Aatalo et al., 1994), compete over the same nest-holes, and also have highly overlapping diets (Chapter I). Previous studies have indicated that pied flycatchers have a broader tolerance of environmental stress (Sætre et al., 1999; Qvarnström et al., 2005), which allows them to persist in habitats of lowest
quality, while being excluded from those of the highest quality (Alatalo et al., 1994). Nevertheless, the overlap in their realized niches (including spatial segregation resulting from competition) is extensive, and long-term persistence of the two species on the Baltic islands is unlikely without further divergence in ecology. Undoubtedly, the persistence of pied flycatchers on the two Swedish study islands is made possible through extensive immigration from surrounding allopatric source populations.

Are hybrids more or less resistant to parasites?

Another component of fitness potentially enhanced by hybridization is resistance against parasites. A high level of heterozygosity across the genome, and in particular among parts coding for the major histocompatibility complex (MHC) may promote overall immunity (Hughes & Hughes, 1995; Wegner et al. 2003; Westerdahl et al., 2005). Furthermore, highly host-specific parasites may be unable to colonise and infect the novel host environment offered by hybrids (Whitham et al., 1994). In Chapter II, I investigate whether hybrid flycatchers are more or less susceptible to infection by blood parasites (*Haemoproteus* and *Plasmodium*) and whether this susceptibility is correlated with their immune-response to a novel antigen, phytohaemagglutinin (PHA). F1 hybrids had parasite infection rates that were intermediate between, but not significantly different from, collared and pied flycatchers (see Figure 4). Furthermore, their immune response to PHA was similar to that of the parents. Later generation hybrids, however, had significantly stronger immune responses than the parental species (Figure 4). They also had the lowest malarial infection rate, although this was not significantly lower than the parental species. More samples are required before it is known whether this pattern is robust.
Figure 4. Rates of infection by avian malarial parasites (left panel) and immune response to PHA (right panel) of collared and pied flycatchers and their first- (F1) and later-generation (RECOM) hybrids (see Chapter II).

In general, this study failed to support the idea that parasite resistance is enhanced in F1 hybrids. This finding agrees with a number of recent studies indicating that parasite resistance of animal hybrids does not tend to be higher than that of the parental species (Moulia, 1999; Derothe et al., 2001; Parris, 2004; Wolinska et al., 2004). This is a comparable pattern to that observed in plants (Moulia, 1999; Fritz et al., 1999), despite vastly different immune systems.

Summarizing selection against hybridization

In flycatchers, individual components of fitness are differently affected by hybridization. Fertility is reduced in F1 hybrids, but their immunity against malarial parasites is not impaired (Chapter II). Furthermore, growing up in a territory possessed by heterospecific parents is sometimes associated with direct benefits (Chapter I). Selection on flycatchers to avoid hybridization is therefore unclear, and may be lower than previously thought, based on fertility alone. In Chapter III, I attempt to resolve this confusion by producing a single estimate of the selection against hybridization; a composite of all individual fitness components. As mentioned earlier, estimating the fitness of hybridizing individuals is a complex problem. Not only is it difficult to find proxies of fitness that accurately reflect genetic representation in later generations, but it is unclear how many generations in the future we should consider. Because of the frequent indirect costs/benefits of hybridization (i.e. hybrid offspring with especially unfit or vigorous hybrid offspring), esti-
mates of a hybridizing individual’s fitness as their genetic contribution to the next generation are unsuitable, as this may not represent their contribution to ‘future’ generations. This is widely recognized, and is why selection against hybridization is typically inferred from the reproductive output of F₁ hybrids, rather than the reproductive output of hybridizing individuals themselves (see recent examples by Parris et al. 1999; Bierne et al., 2002; Pertl et al., 2002; Ramsey et al., 2003; Bleeker & Matthies, 2005; Kirk et al. 2005; Peterson et al., 2005). However, the fitness consequences of hybridization (hybrid vigour or outbreeding depression) may be expressed over several generations (Rieseberg & Carney, 1998; Barton, 2001; Burke & Arnold, 2001). As a result, the indirect costs or benefits of hybridization may be underestimated when only F₁ offspring are considered. Due to the difficulty of estimating fitness in nature, as well as correctly identifying late-generation hybrids, very little is currently known about for how many generations of hybrid offspring the indirect costs of hybridization typically persist. Yet, this information is important if we are to understand how well single- and two-generation estimates of fitness (i.e. based on the reproductive success of hybridizing pairs and their F₁ offspring) represent overall selection against hybridization.

Until recently, the lack of genetic tools for accurately identifying late-generation hybrid flycatchers has prohibited the estimation of selection against hybridization using multiple generations of hybrid descendents. However, recently identified SNPs (Borge et al., 2005) now allow us to identify F₁ hybrids, first-generation backcrosses, and second-generation backcrosses. In Chapter III, I use the number of descendents three generations after hybridization to estimate selection against hybridization. Two independent methods for estimating this (comparing frequencies of the hybrid descendents and combining reproductive data from all hybrid generations) revealed similar results. Hybridizing individuals produce approximately 2% of the number of descendents arising from non-hybridizing individuals. Furthermore, this severe postzygotic isolation between collared and pied flycatchers is likely to be a conservative estimate. This is because I also observed a high rate of ‘re-hybridization’ (mating with the species that constitutes the minority of the individual’s genome) among second-generation backcrosses. This suggests that the depressed fitness of hybrids persists beyond first-generation backcrosses.

Strong postzygotic isolation between collared and pied flycatchers is not surprising considering they have remained distinct in the face of extensive hybridization. Still, this was only detected once multiple generations of hybrid descendents were considered. Chapter III highlights the importance of taking a holistic approach when estimating fitness. This study suggests a crucial role for post-zygotic isolation as a reproductive barrier between newly evolved bird species. This opposes previous suggestions that the evolution of behavioural barriers to interbreeding typically precedes the evolu-
tion of postmating isolation in birds (Grant & Grant, 1997). Continued developments in the genetic tools available for identifying later-generation hybrids (backcrosses and ‘re-hybrids’) will greatly assist in quantifying postzygotic isolation in other study-systems, allowing more educated conclusions to be drawn regarding the evolution of reproductive barriers.
Why does hybridization occur?

Given that selection against hybridization between flycatcher species is so strong, it is worth considering why complete pre-mating isolation has not evolved. Perhaps the two taxa have simply not spent a sufficient amount of time in sympatry for this to occur. Alternatively, other factors may have prevented the evolution of complete pre-mating isolation though reinforcement. Whether reinforcement is common has been a topic of much debate (Bigelow, 1965; Spencer et al., 1986; Butlin, 1987, 1989; Noor, 1999; Coyne & Orr, 2004). Coyne and Orr (2004) highlight the main objections, and I summarise these below. First, gene flow causes recombination among genes coding for assortative mating and genes coding for low hybrid fitness. This often leads to a breakdown in isolation barriers. Second, if one of the two taxa is rare, they suffer higher rates of hybridization. When this is costly, as is required for reinforcement, the rare species is likely to go extinct before pre-mating isolation has evolved. Third, gene flow from outside the hybrid zone prevents local adaptation to sympatry (including the evolution of species recognition). Fourth, as reinforcement progresses, hybridization becomes more infrequent, and this relaxed selection slows further evolution of traits influencing hybridization.

To gain insight into which of these might be important in flycatcher hybrid zones, it is important to examine which factors currently affect hybridization rates. Given that hybridization is costly, there are two main reasons why an individual might still hybridize. First, it may be poorly able to distinguish conspecific mates from heterospecific mates (i.e. there are errors in species recognition). Such a scenario arises if reinforcement has not yet occurred. Alternatively, there may be no errors in species recognition, but conspecific mates are simply not available, forcing hybridization. Thus, hybridization may result even if reinforcement has occurred. Chapters IV and V explore these possibilities in flycatchers.

A lack of conspecific mates

Even if both species display perfect preferences for their own kind, hybridization may still occur when conspecific mates are lacking. If this is the pri-
mary reason for hybridization, we can make a number of predictions. First, the risk of hybridization experienced by individual females increases as their species constitutes a smaller proportion of the population. Second, maximum hybridization rates occur when one species is rare (Randler, 2002). Third, because females are thought to be the choosy sex, heterospecific pairs should mostly contain a female of the rare species (Wirtz, 1999). In Chapter IV, I test each of these predictions in flycatchers. Because the study islands consist of a large number of separate forests with differing ratios of the two species (see Figure 1) and females generally sample potential partners within the one woodlot (Dale & Slagsvold, 1996), I was able to determine how hybridization patterns varied with the relative abundance of the two species.

In accordance with expected effects of lacking conspecific mates, female pied flycatchers had much higher probabilities of hybridizing in sites where conspecifics were rare. A lack of data on female collared flycatchers in pied-dominated sites prevented a similar comparison being done for them, but the available data was consistent with such a pattern (see Figure 5).

![Figure 5](image_url)

*Figure 5.* The effect of relative abundance on the hybridization risk of individual females of either species (see Chapter IV). Each pair of points (collared and pied) represents one or several forest sites with similar species composition.

However, the overall hybridization rate was highest in sites where the two species were at equal frequencies (Figure 6a). This pattern resulted from the fact that, while individual females had lower hybridization risks when they became more common, there were more of them. Simulations confirmed that
such a pattern is consistent with hybrid zones in which there are moderate levels of error in species recognition. This implies that a lack of conspecific mates, while important, is not the sole explanation of hybridization in flycatchers.

This conclusion was further supported by asymmetries in hybridization towards heterospecific pairs involving females of the rare species. Flycatcher hybrid zones in fact showed the opposite pattern; that heterospecific pairs more often contained female pied flycatchers as pied flycatchers were increasingly common (figure 6b). Such a pattern implies that rarity can influence hybridization in ways other than through limiting conspecific mates. Rarity can affect the error rate of choosy females, either through increasing rates of mixed singing (false male signals) or as a plastic response of females to their perceived hybridization risk.

Figure 6. The relationships between relative abundance of pied flycatchers within a forest site and (a) the overall hybridization rate within a site, and (b) the bias in the direction of hybridization towards heterospecific pairs involving female pied flycatchers (see Chapter IV for details).

Overall, much of the hybridization occurring in flycatchers results from a lack of conspecific mates. However, even when the choice between a heterospecific and conspecific is available, errors do occur with notable frequency.
Errors in species recognition

Species recognition depends upon the existence of traits that characterize each species, and preferences of individuals for those traits that signal conspecifics. Errors in mate choice (i.e. hybridization) can therefore result if the traits are not sufficiently divergent, and/or if the preferences for the trait are weak. Chapters IV and V examine components of the species recognition process in flycatchers.

In flycatchers, there is a slight but significant bias towards hybridization mostly involving female pied flycatchers and male collared flycatcher (Chapter IV). From figure 6b, I conclude that this bias probably doesn’t result from pied flycatchers being the generally rarer species across the field sites. I therefore tested whether differences between the females of the two species in their discriminatory ability are a likely reason for this asymmetrical hybridization. In Chapter IV, I present results from a song-broadcast experiment, which indicated that collared flycatchers respond more strongly to conspecific song than pied song. Pied flycatchers, on the other hand, do not display preferences for conspecific song. These differences in discriminatory ability are a likely cause of asymmetrical hybridization. However, the selection on such preferences to be refined within the hybrid zone is dependent on the reliability of the information contained within the song-signal. A large proportion of male pied flycatchers within the hybrid zone copy the songs of male collared flycatchers (Gelter, 1987; Alatalo et al., 1990; Haavie et al., 2004), increasing their probability of being chosen by female collared flycatchers (Qvarnström et al., 2006), and reducing the selection on female pied flycatchers to narrow their preference.

Pied flycatchers within the Swedish hybrid zone have probably lived alongside collared flycatchers for a shorter time than Swedish collared flycatchers have lived alongside pieds. Furthermore, there is probably substantial immigration into the sympatric population of pied flycatchers from allopatric areas (Alatalo et al., 1982). Both factors make it likely that if one of the two species were less adapted to sympatry, it is expected to be the pied flycatcher. Indeed, they show weaker preferences for their own kind, and more often falsely signal their specific identity through song. Comparisons with allopatric populations of collared flycatchers are required to confirm whether their apparent adaptation to sympatry is a result of reinforcement. Migration from allopatric populations is typically implicated as a factor inhibiting reinforcement, and this may be true for pied flycatchers. However, it can also enhance the process in collared flycatchers, by prolonging the time before the local extinction of the competitively least fit taxon.

However, some taxa that have spent a long time in sympatry and experience very little gene flow from allopatry may continue to possess traits that increase their hybridization rate, especially if lacking those traits is associated with costs. In Chapter V, I show that delaying the maturation of secon-
Primary sexual characters until two years of age in collared flycatchers is associated with an increased risk of hybridization among one-year-olds (subadults). This arises because subadults approach pied flycatchers in morphology (see figures 2 and 7), and they are therefore chosen by female pied flycatchers faced with a lack of conspecific mates. Despite this cost of delayed maturation, it persists within the hybrid zone. The maturation of sexual ornaments in flycatchers may be constrained developmentally (as appears to be the case in shorebirds: Chu, 1994), such that it cannot be avoided without substantial re-organization of the developmental process. Alternatively, the benefits associated with reduced aggressive confrontation by older, dominant males (as indicated by Qvarnström, 1997) may override the risk of hybridization in a hybrid zone where heterospecific females are scarce. In either case, the trait increasing hybridization (delayed maturation) is maintained because the costs of lacking it are too great. This highlights an important criticism of speciation via reinforcement; namely, once hybridization becomes rare, further selection on traits that prevent it is weak, and such traits are unlikely to evolve if there are costs or constraints associated with them.

![Figure 7. Principal components analysis of four plumage traits, showing phenotypic overlap between male pied flycatchers (triangles), subadult male collared flycatchers (squares) and adult male collared flycatchers (crosses) (see Chapter V).](image)
This study provides one of the first comprehensive estimates of the strength of selection against natural hybridization. It also highlights that different components of fitness can be differently affected by hybridization, such that it is rarely possible to know a priori if any particular component acts as an appropriate proxy for overall selection. By estimating fitness in a context that is biologically relevant (i.e. in nature), studies such as presented in this thesis provide a basis for inferring the range of conditions under which populations fuse or remain separate in the face of hybridization. This knowledge is fundamental for understanding and managing the diversity of life on Earth. With continuing advances in the genetic tools available for identifying later-generation hybrids and monitoring gene flow, estimating the strength of selection against hybridization may soon be an achievable goal in many other hybrid zones.

I found that post-mating barriers play a crucial role in maintaining species integrity in flycatchers, while pre-mating barriers are incomplete. This contrasts with previous suggestions that pre-mating barriers typically evolve fastest in birds (Grant & Grant, 1997). Clearly, the relative importance for speciation of pre- versus post-mating barriers to gene flow remains unclear. Investigations into the number of genes typically involved in such barriers may give insight into their likely rate of evolution, and therefore importance.

Strong selection against hybridization need not always result in reinforced pre-mating isolation. I identify a number of traits (e.g. song discrimination and copying, delayed maturation of sexual ornaments) influencing hybridization, implying that these traits are under different selection within and outside the hybrid zone. This thesis therefore provides testable predictions about the expected directions of change in the values of these traits between allopatric and sympatric populations of each species, as predicted from reinforcement. Such comparative studies will provide an indication of the role that reinforcement has had in causing the pre-mating isolation currently observed within the flycatcher hybrid zone.

Finally, this thesis highlights a generally neglected reason for hybridization; a lack of conspecific mates. Even when individuals express perfect preferences for their own kind, hybridization may occur when no conspecific mates are available to choose from. Whether this is a widespread phenomenon is not known, but some studies suggest that it is (Randler, 2002). In hybrid zones where it is more beneficial to hybridize than to not breed at all,
hybridization may occur as an adaptive “back-up” strategy. This may have important repercussions for reinforcement, and yet this is rarely considered.

Overall, the *Ficedula* hybrid zone provides an ideal opportunity for studying a range of questions pertaining to speciation, and this thesis provides an important early step towards answering some of these.


Jag har fokuserat på två huvudfrågeställningar:
(1) Hur stark är den evolutionära urvalsprocessen (selektionen) emot hybridisering och hur kan man bäst uppskatta och mäta den?

(2) Varför förkommer hybridisering när det leder till avkommor med nedsatt fortplantningsförmåga?

ÄR HYBRIDISERING KOSTSAMT?


En annan tänkbar fördel kopplat till hybridisering är immunitet mot parasiter. Hybrider har mer variation i sina gener jämfört med föräldrarterna, vilket inkluderar gener som styr immunförsvaret. Ökad variation bland dessa gener kan leda till bättre immunförsvar genom motståndskraft mot fler typer av parasiter. Dessutom skulle hybrider kunna undslippa många parasiter genom att helt enkelt utgöra en miljö som den vissa parasiter inte är anpassad till. Många parasiter är nämligen ofta starkt anpassade till specifika världorganismer. I kapitel II undersöker jag om hybridflugsnappare har relativt färre blodparasiter och ett starkare immunförsvar. Jag fann att hybriders immunförsvar var intermediärt och inte skiljde sig från någon av föräldrarterna. Infektionsfrekvensen av blodparasiter skiljde sig däremot mellan de båda föräldrarterna och halsbandsflugsnappare hade färre parasiter. Detta kan, i sin tur, påverka hur pass konkurrenskraftiga de båda arterna är och därmed påverka deras samexistens i hybridzonen.

En viktig slutsats som man kan dra från flugsnapparna är att olika processer under deras liv kan påverkas olika av hybridisering. Hybriders fertilitet är nedsatt men inte deras förmåga att motstå parasiter. Dessutom kan

VARFÖR SKER HYBRIDISERING?


längre ett viktigt selektionstryck och utvecklingen mot en fullständig repro-
duktiv isolering avstannar.

SLUTSATSER
Den här avhandlingen presenterar det hitintills bästa måttet på styrkan av
selektionen mot hybridisering i en naturlig population. Genom att undersöka
selektion mot hybridisering i en naturlig miljö kan studier som min ta reda
på vilka förhållande som gör att populationer som hybridiserar antingen slås
samman eller separerar från varandra. Den typen av kunskap är kritisk för
vår förståelse av uppkomsten och bevarandet av biodiversitet på jorden. Ut-
vecklingen av genetiska hjälpmedel gör det realistiskt att spåra hybridisering
i flera generationer och i mer detalj studera genflöde mellan populationer i
hybridzoner även hos andra arter än flugsnappare.

Jag fann att **postzygotiska barriärer** mot genflöde mellan flugsnappararterna är starka medan de **prezygotiska bärriärerna** är förhållandevis
ofullkomliga. Den upptäckten kontrasterar med den tidigare uppfattningen
att **prezygotiska bärriärer** generellt utvecklas snabbare hos fåglar. Jag identi-
fierade ett antal karaktärer (t.ex. förmåga att särskilja den egna artens sång,
sångkopiering, åldersrelaterad fjäderdräkt) som ger upphov till en ofullkom-
lig **prezygotisk barriär**. I förlängningen leder mina resultat till testbara
förutsägelser om hur dessa karaktärer kommer att utvecklas i populationer
som lever inom och utanför hybridzonen. Sådana jämförelser skulle kunna
avslöja om reproduktiv isolering delvis utvecklas till följd av direkt selektion
för ökad isolering i hybridzoner eller om den uppstår som en sidoeffekt av
processer som helt sker inom arten.

**Ficedula** hybridzonen är ett ypperligt studiesystem för en rad
frågeställningar rörande artbildning, och min avhandling representerar ett
viktigt första steg för att besvara några av dessa centrala frågor om arters
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